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Ambient Water Quality Criteria for Benzene

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16. ABSTRACT

Section 304(A) of the Clean Water Act [33 U.S.C. 1314(A)], requires EPA to publish and periodically update water quality criteria. These criteria are to reflect the latest scientific knowledge on the identifiable effects of pollutants on public health and welfare, aquatic life, and recreation. This report presents water quality criteria for Benzene. It presents concentration criteria for the protection of freshwater and saltwater aquatic life. It presents 'safe' concentrations for humans, and in the case of suspect or proven carcinogens, gives various levels of incremental cancer risk. A Section 304(A) water quality criterion is a qualitative or quantitative estimate of the concentration of a water constituent or pollutant in ambient waters which, when not exceeded, will ensure a water quality sufficient to protect a specified water use. Under the Act a criterion is a scientific entity, based solely on data and scientific judgment. It does not reflect considerations of economic or technological feasibility nor is it a water quality standard and in itself has no regulatory effect.

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BENZENE

Prepared By
U.S. ENVIRONMENTAL PROTECTION AGENCY

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FOREWORD

Section 304 (a)(1) of the Clean Water Act of 1977 (P.L. 95-217), requires the Administrator of the Environmental Protection Agency to publish criteria for water quality accurately reflecting the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare which may be expected from the presence of pollutants in any body of water, including ground water. Proposed water quality criteria for the 65 toxic pollutants listed under section 307 (a)(1) of the Clean Water Act were developed and a notice of their availability was published for public comment on March 15, 1979 (44 FR 15926), July 25, 1979 (44 FR 43660), and October 1, 1979 (44 FR 56628). This document is a revision of those proposed criteria based upon a consideration of comments received from other Federal Agencies, State agencies, special interest groups, and individual scientists. The criteria contained in this document replace any previously published EPA criteria for the 65 pollutants. This criterion document is also published in satisifaction of paragraph 11 of the Settlement Agreement in Natural Resources Defense Council, et. al. vs. Train, 8 ERC 2120 (D.D.C. 1976), modified, 12 ERC 1833 (D.D.C. 1979).

The term "water quality criteria" is used in two sections of the Clean Water Act, section 304 (a)(1) and section 303 (c)(2). The term has a different program impact in each section. In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. The criteria presented in this publication are such scientific assessments. Such water quality criteria associated with specific stream uses when adopted as State water quality standards under section 303 become enforceable maximum acceptable levels of a pollutant in ambient waters. The water quality criteria adopted in the State water quality standards could have the same numerical limits as the criteria developed under section 304. However, in many situations States may want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns before incorporation into water quality standards. It is not until their adoption as part of the State water quality standards that the criteria become regulatory.

Guidelines to assist the States in the modification of criteria presented in this document, in the development of water quality standards, and in other water-related programs of this Agency, are being developed by EPA.

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Office of Water Regulations and Standards

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CRITERIA DOCUMENT

BENZENE

CRITERIA

Aquatic Life

The available data for benzene indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 5,300 μg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of benzene to sensitive freshwater aquatic life.

The available data for benzene indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 5,100 μg/l and would occur at lower concentrations among species that are more sensitive than those tested. No definitive data are available concerning the chronic toxicity of benzene to sensitive saltwater aquatic life, but adverse effects occur at concentrations as low as 700 μg/l with a fish species exposed for 168 days.

Human Health

For the maximum protection of human health from the potential carcinogenic effects due to exposure of benzene through ingestion of contaminated water and contaminated aquatic organisms, the ambient water concentrations should be zero based on the non-threshold assumption for this chemical. However, zero level may not be attainable at the present time. Therefore, the levels which may result in incremental increase of cancer risk over the lifetime are estimated at 10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria are 6.6 μg/l, 0.66 μg/l, and 0.066 μg/l, respectively. If the above estimates are made for consumption of aquatic organisms only, excluding consumption of water, the levels are 400 μg/l, 40.0 μg/l, and 4.0 μg/l, respectively.

INTRODUCTION

Benzene is a volatile, colorless, liquid hydrocarbon produced principally from coal tar distillation and from petroleum by catalytic reforming of light naphthas from which it is isolated by distillation or solvent extraction (Weast, 1972; Ayers and Muder, 1964; U.S. EPA, 1976). It is also produced in coal processing and coal coking operations. The broad utility spectrum of benzene (commercially sometimes called "Benzol") includes its use as: an intermediate for synthesis in the chemical and pharmaceutical industries including the manufacture of styrene, cyclohexane, detergents, and pesticides, a thinner for lacquer, a degreasing and cleaning agent, a solvent in the rubber industry, an antiknock fuel additive, a general solvent in laboratories, a solvent for industrial extraction and rectification, and in the preparation and use of inks in the graphic arts industries.

In the United States today, benzene is used extensively (over 4 million metric tons annually) in the chemical industry and its use is expected to increase when additional production facilities become available (Fick, 1976).

Benzene has the molecular formula C_6H_6 and a molecular weight of 78.1 (Weast, 1972; Ayers and Muder, 1964). Pure benzene has a boiling point of 80.1°C and a melting point of 5.5°C (Weast, 1972). Benzene has a density less than that of water (0.87865 at 20°C) (Weast, 1972; Stecher, 1968).

The solubility and volatile nature of benzene indicate possible environmental mobility. Benzene has been detected at various concentrations in lakes, streams, and finished drinking water. Benzene has been detected in finished drinking water (U.S. EPA, 1975), in water and sediments samples from the lower Tennessee River in ppb concentrations (Goodley and Gordon, 1976) and in the atmosphere (Howard and Durkin, 1974).

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Aquatic Life Toxicology*

INTRODUCTION

Most of the toxicity data concerning the effects of benzene on aquatic life have been determined using static test conditions without measured concentrations. Consequently, these data may underestimate the toxicity of this volatile chemical. Nearly all of the adverse acute and chronic effects occurred at concentrations above 5,000 µg/l. However, some data (Struhsaker, 1977) in Table 5 indicate that unique acute effects may occur at benzene concentrations as low as 700 µg/l.

EFFECTS

Acute Toxicity

Two freshwater invertebrate species, *Daphnia magna* and *Daphnia pulex*, have been tested using static conditions (U.S. EPA, 1978; Canton and Adema, 1978). The 48-hour effect concentrations ranged from 203,000 to 620,000 µg/l (Table 1). The species acute values for *Daphnia magna* and *Daphnia pulex* are 380,000 and 300,000 µg/l which result indicates no appreciable difference in sensitivity.

Six freshwater fish species representing four families have been tested with benzene, and the 96-hour LC_{50} values ranged from 5,300 µg/l for the rainbow trout under flow-through test conditions with measured concentrations to 386,000 µg/l for the mosquito fish (Table 1). Because of the difference in test methods for the rainbow trout and the other five species, on which tests were conducted under static conditions without measured concentrations, one cannot conclude whether this difference is due to different sensitivity or test methods. Two additional non-standard tests with salmonid species yielded LC_{50} and LC_{30} results of 12,000 and 15,100 µg/l (Table 5).

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*The reader is referred to the Guidelines for Deriving Water Quality Criteria for the Protection of Aquatic Life and Its Uses in order to better understand the following discussion and recommendation. The following tables contain the appropriate data that were found in the literature, and at the bottom of each table are calculations for deriving various measures of toxicity as described in the Guidelines.

Several saltwater invertebrate and one fish species have been studied (Table 1). There was quite a bit of variability among the invertebrate species with a range of effect concentrations of 17,600 to 924,000 µg/l. The striped bass was more sensitive with 96-hour LC_{50} values of 10,900 and 5,100 µg/l.

Potera (1975) conducted a variety of 24-hour exposures with the grass shrimp, *Palaemonetes pugio*, using static procedures with measured concentrations (Table 5). Temperature (10 and 20°C), salinity (15 and 25 ppt), and life stage (larvae and adults) were the variables considered. The total range of LC_{50} values for the six tests was 33,500 to 90,800 µg/l which small difference indicates that the variables did not have a very great effect. This difference in salinity was also evaluated for the copepod, *Nitocra spinipes*, and the 24-hour LC_{50} values were 82,000 µg/l at 15 ppt salinity and 111,500 µg/l at 25 ppt (Table 5).

In both freshwater and saltwater systems, the fish species appear to be more sensitive than the invertebrate species.

Chronic Toxicity

A chronic test with *Daphnia magna* was conducted (U.S. EPA, 1978) but the results were incomplete. No adverse effects were observed at test concentrations as high as 98,000 µg/l (Table 2). It is interesting to note that the species acute value for this species is 380,000 µg/l (Table 1) which indicates only a relatively small difference between the acute and chronic effects of benzene on this species.

No chronic toxicity data are available for any freshwater or saltwater species.

A summary of species acute and chronic values is listed in Table 3.

Plant Effects

Kauss and Hutchinson (1975) determined that there was a 50 percent reduction in the cell numbers of *Chlorella vulgaris* after 48 hours at a concentration of 525,000 µg/l (Table 4).

Three saltwater algal or diatom species have been tested (Dunstan et al., 1975; Atkinson et al., 1977) and growth was inhibited at benzene concentrations of 20,000 to 100,000 µg/l (Table 4).

Residues

No measured steady-state bioconcentration factor is available for benzene.

Miscellaneous

The 96-hour LC_{30} for the fathead minnow using flow-through methods with measured concentrations was 15,100 µg/l (Table 5), which result is not too different from the static test results for the 96-hour LC_{50} values of 33,470 and 32,000 µg/l (Table 1).

Struhsaker (1977) exposed female Pacific herring to 700 µg/l for 48 hours just prior to their spawning. Survival of embryos at hatching and survival of larvae upon continued exposure through yolk absorption were reduced (Table 5). The results of this study need further verification before such effects may be used to derive a criterion for saltwater organisms. Only one test concentration was used to determine the effects of benzene on embryo survival and survival of larvae; a no-effect concentration was not determined. Also the adult fish were captured from San Francisco Bay waters which, as stated by the authors, may affect the hatchability of Pacific herring eggs due to the effects of accumulated pollutants in the adults' gonads.

The data by Potera (1975) were discussed earlier.

Summary

The acute toxicity of benzene to freshwater species has been measured with eight species and the species acute values range from 5,300 µg/l to 386,000 µg/l. No data are available for benthic crustaceans, benthic insects, or detritivores. However, the most important deficiency may be that only with the rainbow trout were the results obtained from a flow-through test and based on measured concentrations. Results based on unmeasured concentrations in static tests are likely to underestimate toxicity for compounds like benzene that are relatively volatile.

A life cycle test was conducted with one freshwater species, Daphnia magna, but no concentration up to 98,000 µg/l caused an adverse effect. On the other hand, concentrations which apparently did not adversely affect Daphnia magna in a life cycle test did affect other species in acute tests.

For saltwater species, species acute values are available for one fish species and five invertebrate species and range from 10,900 to 924,000 µg/l. These values suggest that saltwater species are about as sensitive as freshwater species. The one acute value from a flow-through test in which toxicant concentrations were measured was not the lowest value, as was the case with the freshwater acute data. Saltwater plants seem to be about as sensitive as saltwater animals. Other data indicate that herring may have suffered stress and some mortality at 700 µg/l.

CRITERIA

The available data for benzene indicate that acute toxicity to freshwater aquatic life occurs at concentrations as low as 5,300 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No data are available concerning the chronic toxicity of benzene to sensitive freshwater aquatic life.

The available data for benzene indicate that acute toxicity to saltwater aquatic life occurs at concentrations as low as 5,100 µg/l and would occur at lower concentrations among species that are more sensitive than those tested. No definitive data are available concerning the chronic toxicity of benzene to sensitive saltwater aquatic life but adverse effects occur at concentrations as low as 700 µg/l with a fish species exposed for 168 days.

Table 1. Acute values for benzene

Species	Method*	LC50/EC50 (µg/l)	Species Mean Acute Value (µg/l)	Reference
		FRESHWATER SPECIES		
Cladoceran, *Daphnia magna*	S, U	203,000	-	U.S. EPA, 1978
Cladoceran, *Daphnia magna*	S, U	400,000	-	Canton & Adema, 1978
Cladoceran, *Daphnia magna*	S, U	620,000	-	Canton & Adema, 1978
Cladoceran, *Daphnia magna*	S, U	412,000	-	Canton & Adema, 1978
Cladoceran, *Daphnia magna*	S, U	412,000	-	Canton & Adema, 1978
Cladoceran, *Daphnia magna*	S, U	356,000	-	Canton & Adema, 1978
Cladoceran, *Daphnia magna*	S, U	356,000	380,000	Canton & Adema, 1978
Cladoceran, *Daphnia pulex*	S, U	345,000	-	Canton & Adema, 1978
Cladoceran, *Daphnia pulex*	S, U	265,000	300,000	Canton & Adema, 1978
Rainbow trout (juvenile), *Salmo gairdneri*	FT, M	5,300	5,300	DeGraeve, et al. 1980
Goldfish, *Carassius auratus*	S, U	34,420	34,000	Pickering & Henderson, 1966
Fathead minnow, *Pimephales promelas*	S, U	33,470	-	Pickering & Henderson, 1966
Fathead minnow, *Pimephales promelas*	S, U	32,000	33,000	Pickering & Henderson, 1966

Table 1. (Continued)

Species	Method*	LC50/EC50 (µg/l)	Species Mean Acute Value (µg/l)	Reference
Guppy, Poecilia reticulata	S, U	36,600	36,600	Pickering & Henderson, 1966
Mosquitofish, Gambusia affinis	S, U	386,000	386,000	Wallen, et al. 1957
Bluegill, Lepomis macrochirus	S, U	22,490	22,000	Pickering & Henderson, 1966
		SALTWATER SPECIES		
Pacific oyster, Crassostrea gigas	S, U	924,000	924,000	LeGore, 1974
Copepod, Tigriopus californicus	S, M	450,000	450,000	Korn, et al. 1976
Bay shrimp, Crago franciscorum	S, M	17,600	17,600	Benville & Korn, 1977
Grass shrimp, Palaemonetes pugio	S, U	27,000	27,000	Tatem, 1975
Dungeness crab (larva), Cancer magister	S, U	108,000	108,000	Caldwell, et al. 1977
Striped bass, Morone saxatilis	FT, M	10,900	-	Meyerhoff, 1975
Striped bass, Morone saxatilis	S, M	5,100	10,900	Benville & Korn, 1977

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* S = static, FT = flow-through, U = unmeasured, M = measured

Table 2. Chronic values for benzene (U.S. EPA, 1978)

Species	Test*	Limits (µg/l)	Chronic Value (µg/l)
	FRESHWATER SPECIES		
Cladoceran, *Daphnia magna*	LC	>98,000	>98,000

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* LC = life cycle or partial life cycle

Table 3. Species mean acute and acute-chronic ratios for benzene

Rank*	Species	Species Mean Acute Value (µg/l)	Acute-Chronic Ratio
		FRESHWATER SPECIES	
8	Mosquitofish, *Gambusia affinis*	386,000	-
7	Cladoceran, *Daphnia magna*	380,000	-
6	Cladoceran, *Daphnia pulex*	300,000	-
5	Guppy, *Poecilia reticulata*	36,600	-
4	Goldfish, *Carassius auratus*	34,000	-
3	Fathead minnow, *Pimephales promelas*	33,000	-
2	Bluegill, *Lepomis macrochirus*	22,000	-
1	Rainbow trout, *Salmo gairdneri*	5,300	-
		SALTWATER SPECIES	
6	Pacific oyster, *Crassostrea gigas*	924,000	-
5	Copepod, *Tigriopus californicus*	450,000	-
4	Dungeness crab, *Cancer magister*	108,000	-
3	Grass shrimp, *Palaemonetes pugio*	27,000	-

Table 3. (Continued)

Rank*	Species	Species Mean Acute Value (µg/l)	Acute-Chronic Ratio
2	Bay shrimp, *Crago franciscorum*	17,600	-
1	Striped bass, *Morone saxatilis*	10,900	-

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* Ranked from least sensitive to most sensitive based on species mean acute value.

Table 4. Plant values for benzene

Species	Effect	Result (µg/l)	Reference
	FRESHWATER SPECIES		
Alga, *Chlorella vulgaris*	48-hr EC50 50% reduction in cell numbers	525,000	Kauss & Hutchinson, 1975
	SALTWATER SPECIES		
Dinoflagellate, *Amphidinium carterae*	Growth inhibition	>50,000	Dunstan, et al. 1975
Diatom, *Skeletonema costatum*	Growth inhibition	100,000	Dunstan, et al. 1975
Diatom, *Skeletonema costatum*	Growth inhibition	20,000	Atkinson, et al. 1977
Alga, *Cricosphaera carterae*	Growth inhibition	50,000	Dunstan, et al. 1975

Table 5. Other data for benzene

Species	Duration	Effect	Result (µg/l)	Reference
		FRESHWATER SPECIES		
Brown trout, *Salmo trutta*	1 hr or 24 hrs	LC50	12,000	Woodiwiss & Fretwell, 1974
Fathead minnow, *Pimephales promelas*	96 hrs	LC30	15,100	DeGraeve, et al. 1980
		SALTWATER SPECIES		
Copepod, *Nitocra spinipes*	24 hrs	LC50	82,000	Potera, 1975
Copepod, *Nitocra spinipes*	24 hrs	LC50	111,500	Potera, 1975
Grass shrimp (adult), *Palaemonetes pugio*	24 hrs	LC50	38,000	Potera, 1975
Grass shrimp (adult), *Palaemonetes pugio*	24 hrs	LC50	33,500	Potera, 1975
Grass shrimp (adult), *Palaemonetes pugio*	24 hrs	LC50	40,200	Potera, 1975
Grass shrimp (adult), *Palaemonetes pugio*	24 hrs	LC50	40,800	Potera, 1975
Grass shrimp (larva), *Palaemonetes pugio*	24 hrs	LC50	90,800	Potera, 1975
Grass shrimp (larva), *Palaemonetes pugio*	24 hrs	LC50	74,400	Potera, 1975
Pacific herring, *Clupea harengus pallasi*	144 hrs	Stress observed	700	Struhsaker, 1977
Pacific herring, *Clupea harengus pallasi*	168 hrs	Survival reduction	700	Struhsaker, 1977
Striped bass, *Morone saxatilis*	168 hrs	Temporary weight reduction	6,000	Korn, et al. 1976

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Mammalian Toxicology and Human Health Effects

EXPOSURE

Ingestion

Benzene is soluble in water (1,780 mg/l at 25°C) but human exposure through food and water is difficult to quantify because of a relative paucity of data. Four of ten water supplies surveyed by the EPA utilizing volatile organic analysis (VOA) contained benzene at concentrations of 0.1 to 0.3 ug/l; the highest concentration ever reported in a finished water was 10 ug/l (U.S. EPA, 1975; NAS, 1977).

Only limited data on benzene in water are available. A review of benzene sampling data by Howard and Durkin (1974) found that the few freshwater samples analyzed by that time showed only trace levels of benzene. For example, a 1972 EPA study cited in the report identified 53 organic chemicals, ranging from acetone to toluene, in the finished waters and organic waste effluents in 11 plants (of 60 sampled) discharging into the Mississippi River. Benzene was not detected in the effluents, but the trace detected in the finished waters suggested another source than effluent discharge.

A recent sampling of five benzene production or consumption plants by Battelle (1977) found benzene levels in water ranging from <1.0 to 179.0 ppb (plant effluent). The concentrations at 13 upstream and downstream sample locations in nearby receiving waters, however, ranged from <1.0 to 13.0 ppb, with an average of 4.0 ppb.

A recent report by the National Cancer Institute (NCI, 1977) noted benzene levels of 0.1 to 0.3 ppb in four U.S. city drinking water supplies. One measurement from a groundwater well in Jacksonville, Florida showed levels higher than 100 ppb. No indication is given in the report of the sampling methods or the analytical procedures. Howard and Durkin (1974) tabulated environmental monitoring data for benzene in ambient air and water (Table 1).

TABLE 1

Environmental Monitoring Data for Benzene in Ambient Air and Water*

REFERENCE*	TYPE OF SAMPLE	GEOGRAPHICAL LOCATION	SAMPLING METHOD[a]	ANALYSIS TECHNIQUE[a]	QUANTITIES DETECTED
Gordon and Goodley (1971)	Water and mud	Lower Tennessee River	CCE liquid - liquid extract	GC/MS	Not reported
U.S. EPA (1972)	Finished water	Carrollton Plant, New Orleans	CCE	GC	Not attempted
Friloux (1971)	Finished water	U.S. PHS Hospital Carville, La.	CCE	GC	"trace" ppb-ppm range
Novak, et al. (1973)	"Polluted" and "pure" drinking water	Prague, Czecho-slovakia	Inert gas stripping	GC, GC/MS	0.1 ppb
Williams (1965)	Ambient air	Vancouver, Canada	Cold trap - GC column	Rapid heating into GC	1-10 ppb
Smoyer, et al. (1971)	Ambient air	Vicinity of solvent reclamation plant	Grab sample	Direct ingestion into GC; MS, IR	23 ppm
Neligan, et al. (1965)	Ambient air	Los Angeles basin	Cold trap - firebrick	Rapid heating	0.005-0.022 ppm (V/V)

TABLE 1 (continued)

Environmental Monitoring Data for Benzene in Ambient Air and Water*

REFERENCE*	TYPE OF SAMPLE	GEOGRAPHICAL LOCATION	SAMPLING METHOD[a]	ANALYSIS TECHNIQUE[a]	QUANTITIES DETECTED
Altschuller and Bellar (1963)	Ambient air	Downtown Los Angeles	Grab sample	direct injection into GC	0.015-0.06 ppm (V/V)
Lonneman, et al. (1968)	Ambient air	Los Angeles basin	Cold trap - glass beads	rapid heading into GC	aver. 0.015 ppm; highest 0.057 ppm (V/V)
Grob and Grob (1971)	Ambient air	Zurich, Switzerland	Charcoal trap - carbon disulfide extract	GC-MS GC-FL	0.054 ppm
Stephens (1973)	Ambient air	Riverside, California	Cold trap - GC column	GC-FL	0.007-0.008 ppm
Pilar and Graydon (1973)	Ambient air	Toronto, Canada	Cold trap - GC column	GC-FL	aver. 0.013 ppm; highest 0.098 ppm

[a] CCE - carbon chloroform extract, GC - gas chromatography, FL - flame ionization, IR - infrared spectrometry, MS - mass spectrometry.

*Source: Howard and Durkin, 1974.

One possible source of benzene in the aquatic environment is from cyclings between the atmosphere and water (Mitre Corp., 1976). Benzene is fairly volatile (high vapor pressure of 100 mm Hg at 26°C) and has a relatively high solubility (1,780 mg/l at 25°C). Consequently, it is reasonable to believe that benzene could be washed out of the atmosphere with rainfall and then be evaporated back into the atmosphere, causing a continuous recycling between the two media. Benzene is also expected to be photooxidized in air and otherwise biodegraded in the environment.

The exposure to benzene through general dietary intake is not considered to be a problem for the general population. However, benzene has been detected in various food categories: fruits, nuts, vegetables, dairy products, meat, fish, poultry, eggs, and several beverages; an indication of this has been tabulated by the National Cancer Institute (1977) as presented in Table 2. NCI estimated that an individual could ingest as much as 250 ug/day from these foods. The presence of benzene in several other foods has been confirmed by a number of researchers using gas chromatography coupled with mass spectroscopy (Table 3).

The distribution of benzene in the aquatic system is not well documented. Neely, et al. (1974) demonstrated a relationship between octanol/water partition coefficients and bioaccumulation potential in fish. To protect human health, water quality criteria should apply to saltwater as well as freshwater because the major portion of the aquatic life consumed in the United States is obtained from saltwater.

A bioconcentration factor (BCF) relates the concentration of a chemical in aquatic animals to the concentration in the water in which they live. The steady-state BCFs for a lipid-soluble compound in the tissues of various

TABLE 2

Estimated Benzene Levels In Food*

Food	Benzene Level in Food µg/kg
Heat treated or canned beef	2
Jamaican rum	120
Irradiated beef	19
Eggs	500-1900

*Source: NCI, 1977

TABLE 3

Foods Containing Benzene

FOOD	REFERENCES
Haddock fillet	Angelini, et al. 1975
Red beans	Buttery, et al. 1975
Blue cheese	Day and Anderson, 1965
Cheddar cheese	Day and Libbey, 1964
Cayenne pineapple	Flath and Forrey, 1970
Roasted filberts	Kinlin, et al. 1972
Potato tubers	Meigh, et al. 1972
Cooked chicken	Nonaka, et al. 1967
Hothouse tomatoes	Schormuller and Kockmann, 1969
Strawberries	Teranishi, et al. 1963
Black currants	von Sydow and Karlsson, 1971
Roasted peanuts	Waldradt, et al. 1971
Soybean milk	Wilkins and Lin, 1970
Codfish	Wong, et al. 1967

aquatic animals seem to be proportional to the percent lipid in the tissue. Thus the per capita ingestion of a lipid-soluble chemical can be estimated from the per capita consumption of fish and shellfish, the weighted average percent lipids of consumed fish and shellfish, and a steady-state BCF for the chemical.

Data from a recent survey on fish and shellfish consumption in the United States were analyzed by SRI International (U.S. EPA, 1980). These data were used to estimate that the per capita consumption of freshwater and estuarine fish and shellfish in the United States is 6.5 g/day (Stephan, 1980). In addition, these data were used with data on the fat content of the edible portion of the same species to estimate that the weighted average percent lipids for consumed freshwater and estuarine fish and shellfish is 3.0 percent.

No measured steady-state bioconcentration factor (BCF) is available for benzene, but the equation "Log BCF = (0.85 Log P) - 0.70" can be used (Veith, et al. 1979) to estimate the BCF for aquatic organisms that contain about 7.6 percent lipids (Veith, 1980) from the octanol/water partition coefficient (P). Based on an average measured log P value of 2.14 (Hansch and Leo, 1979; Dec, et al., Manuscript), the steady-state bioconcentration factor for benzene is estimated to be 13.2. An adjustment factor of 3.0/7.6 = 0.395 can be used to adjust the estimated BCF from the 7.6 percent lipids on which the equation is based to the 3.0 percent lipids that is the weighted average for consumed fish and shellfish. Thus, the weighted average bioconcentration factor for benzene and the edible portion of all freshwater and estuarine aquatic organisms consumed by Americans is calculated to be 13.2 x 0.395 = 5.21.

Inhalation

The respiratory route is the major source of human exposure to benzene, and much of this exposure is by way of gasoline vapors and automotive emissions. American gasolines contain an average of 0.8 percent (by weight) benzene, and European gasolines contain an average of 5 percent (Goldstein, 1977a). Benzene comprises approximately 2.15 percent (by weight) of the total hydrocarbon emissions from a gasoline engine; this is approximately equivalent to 4 percent benzene (by weight) in automotive exhaust (Howard and Durkin, 1974). This can be extrapolated to an annual benzene emission from automotive exhaust of 940 million pounds in 1971, which is well over one-half of the benzene released to the environment. The geographical distribution of this emission probably approximates population density distribution. Release of benzene into the environment from industrial and commercial use probably does not exceed 30 percent of the total. Other sources are relatively insignificant (Howard and Durkin, 1974).

Concentrations of benzene in the air around gas stations have been found to be 0.3 to 2.4 ppm [National Academy of Sciences (NAS), 1977]. Lonneman, et al. (1968) measured an average concentration of 0.015 ppm in Los Angeles air, with a maximum of 0.057 ppm. The rural background level for benzene has been reported as 0.017 ppb (Cleland and Kingsbury, 1977). Recently, Young, et al. (1978) have brought attention to the fact that consumers may be exposed unknowingly to benzene in the home in the form of paint strippers, carburetor cleaners, denatured alcohol, rubber cement, and art and craft supplies.

Dermal

Since liquid benzene is poorly absorbed through the intact skin [National Institute for Occupational Safety and Health (NIOSH), 1974] and skin contact is infrequent, the dermal route is only a minor source of human exposure.

PHARMACOKINETICS

Absorption

The most frequent route of human exposure to benzene is via inhalation. Toxic effects in humans have often been attributed to combined inhalation and dermal exposure. For example, rotogravure workers were described as washing ink from their hands in open vats of benzene (Hunter, 1962). Although Lazarew, et al. (1931) claimed that benzene could be absorbed by rabbits through the skin, neither Cesaro (1946), nor Conca and Maltagliati (1955) could demonstrate significant percutaneous absorption in humans. Nevertheless, small amounts of benzene absorbed by this route may not have been detected.

Distribution

Benzene accumulated primarily in fatty tissues in the dog (Schrenk, et al. 1941), the mouse (Andrews, et al. 1977) and the rat (Rickert, 1979). Co-administration of toluene with benzene does not alter the accumulation of benzene in the various organs of the mouse (Andrews, et al. 1977). The fat and marrow contained the greatest concentrations of benzene; blood, liver, and kidney also contained significant amounts of benzene; less benzene was observed in the spleen, lung, and brain.

Benzene metabolites were highest in bone marrow and liver. It is noteworthy that in both mice given ^{3}H-benzene by subcutaneous injection (Andrews, et al. 1977), and rats given benzene by inhalation, the concentrations of benzene metabolites in the bone marrow exceeded those in blood. These data taken with the reports of Andrews, et al. (1979) and Irons, et al. (1980) describing the metabolism of benzene in bone marrow preparations, have presented a strong argument to implicate the marrow as the site at which the toxic metabolite(s) of benzene is (are) formed. It is more likely, however, that metabolites coming from the liver are trapped in the marrow because it has been demonstrated that partial hepatectomy prevents benzene-induced bone marrow depression and reduces the accumulation of benzene metabolites in marrow.

In the course of a series of studies on benzene metabolism in vivo it was observed (Snyder, et al. 1978) that metabolites of benzene remained covalently bound to residual protein of liver, brain, kidney, spleen, and fat in mice. Further studies showed that the degree of binding was dose dependent and increased in both liver and bone marrow upon repeated exposure. Tunek, et al. (1978) reported that the covalently bound species in liver microsomes was not likely to be benzene oxide but a metabolite of phenol. Tunek, et al. (1979) have gone on to investigate specific microsomal proteins to which benzene covalently binds. Lutz and Schlatter (1977) reported on the covalent binding of benzene to DNA in liver nuclei. These authors feel that covalent binding to DNA in liver offers a model for the study of the mechanism of benzene toxicity and/or carcinogenesis in bone marrow. This hypothesis may be supported by the report that in the partially hepatectomized rat there was a decrease of not only soluble metabolites in the bone marrow but also of covalent binding (Sammett, 1979).

Metabolism

It has been known since the latter part of the nineteenth century that benzene is biologically converted to phenol (Schultzen and Naunyn, 1867) as well as to catechol and hydroquinone (Nencki and Giocosa, 1880). The first detailed studies of the metabolites of benzene formed in vivo were reported by Porteous and Williams (1949a,b), and with the advent of ^{14}C-benzene these studies were improved upon by Parke and Williams (1953). Extensions of this work in recent years have largely concentrated on metabolism in various animal species, on the mechanism of benzene metabolism using in vitro techniques and on attempting to relate benzene metabolism to its toxicity (Snyder and Kocsis, 1975; Snyder, et al. 1977).

In a landmark series of papers (Porteous and Williams, 1949a,b; Parke and Williams, 1953), outlined the broader aspects of benzene metabolism in rabbits by identifying most of the metabolites in urine as well as those in expired air. He later demonstrated that about one percent could be recovered in bile (Abou-el-Marakem, et al. 1967). The major hydroxylation product was phenol which, along with some catechol and hydroquinone, is found for the most part in urine conjugated with ethereal sulfate or glucuronic acid. Unconjugated phenol has been found in mouse (Andrews, et al. 1977) and rat (Cornish and Ryan, 1965) urine after benzene administration. Parke and Williams (1953) also reported on the occurrence of phenylmercapturic acid and muconic acid. The latter, along with labeled carbon dioxide found in the expired air, suggested that some opening of the ring occurred. Andrews, et al. (1977) estimated that a 25 g mouse could metabolize, at most, approximately 1 mmole of benzene per day.

Benzene metabolism has been studied in liver homogenates (Snyder, et al. 1967; Hirokawa and Nomiyama, 1962; Sakamoto, et al. 1957), cell supernatant fractions containing microsomes (Snyder, et al. 1967; Kocsis, et al. 1968; Sakamoto, et al. 1957; Sato and Nakajima, 1979a,b) and microsomes (Posner, et al. 1961; Snyder, et al. 1967; Gonasun, et al. 1973; Drew, et al. 1974; Harper, et al. 1973; Tunek, et al. 1978). It is clear from these studies that benzene is metabolized in liver microsomes of rat, rabbit, and mouse. Gonasun, et al. (1973) demonstrated that the first step is mediated by the mixed function oxidases. Jerina and co-workers (Jerina, et al. 1968;

Jerina and Daly, 1974) have outlined a pathway for benzene metabolism which revolves about the formation of benzene oxide, an epoxide of benzene, as the first product. This highly unstable intermediate rearranges non-enzymatically to form phenol. This step accounts for the occurrence of phenol as the major metabolite of benzene found in urine. Catechol formation is thought to result from the hydration of benzene oxide by the enzyme epoxide hydratase followed by oxidation to catechol. The intermediate dihydrodiol was observed in rat urine by Sato, et al. (1963). The evidence for the epoxide intermediate is that addition of the epoxide to liver preparations yields the same metabolites as benzene (Jerina, et al. 1968) and the addition of excess hydratase enzyme increases the formation of catechol (Tunek, et al. 1978). Thus, it appears that phenol and catechol are formed by two distinctly different metabolic pathways.

The other dihydroxylated derivative, hydroquinone, is thought to result from a second passage of phenol through the mixed function oxidases. The premercapturic acid, i.e., the glutathione conjugate, is formed by the addition of glutathione to the epoxide via the glutathione transferase enzyme (Jerina, et al. 1968).

The metabolism of benzene in vitro can be altered by the use of enzyme inducing agents administered to the animals prior to sacrifice or by the addition of inhibitors to the incubation mixtures. Benzene (Snyder, et al. 1967), phenobarbital (Snyder, et al. 1967; Drew, et al. 1974), 3-methylcholanthrene (Drew, et al. 1974), and dimethyl sulfoxide (Kocsis, et al. 1968), are all microsomal stimulants for the metabolism of benzene. On the other hand, benzene metabolism can be inhibited by carbon monoxide, aniline, metyrapone, SKF 525A, aminopyrine, cytochrome c (Gonasun, et al. 1973), aminotriazole (Hirokawa and Nomiyama, 1962), and toluene (Andrews, et al.

1977). Gut (1978) has argued that alterations of benzene metabolism *in vitro* by enzyme induction may not be reflective of the overall rate of benzene metabolism in whole animals because the *in vitro* systems do not accurately mimic the pharmacokinetics observed *in vivo*.

The strongest evidence supporting the concept that benzene must be metabolized to produce bone marrow depression is based on the facts that benzene toxicity is prevented by coadministration of toluene, which inhibits benzene metabolism (Andrews, et al. 1977), and that partial hepatectomy protects animals against benzene toxicity while decreasing benzene metabolism (Sammett, 1979). These studies also suggest that despite the fact that benzene is metabolized to some extent in bone marrow (Andrews, et al. 1979; Irons, et al. 1980), the liver must be intact for benzene toxicity to occur. Previous reports of protection against toxicity in phenobarbital treated animals (Ikeda and Ohtsuji, 1971; Drew, et al. 1974) reflect the fact that phenobarbital probably increased the detoxification rate in liver. On the other hand, inhibition of metabolism by toluene and also by aminotriazole (Hirokawa and Nomiyama, 1962) protected animals by decreasing the rate of formation of toxic metabolites. Thus, it appears that a metabolite formed in liver is transported into the marrow where it is converted to a compound which cannot be removed from the marrow and accordingly accumulates (Andrews, et al. 1977; Rickert, et al. 1979) leading to a metabolic impairment expressed as bone marrow depression. Similar mechanisms may play a role in benzene-induced leukemogenesis.

Determination of benzene metabolism in humans was first evaluated as a measure of exposure. Yant, et al. (1936) suggested that since benzene metabolites in the urine could be detected as ethereal sulfates it would be possible to estimate benzene exposure by measuring the ratio of urinary inorganic to organic sulfate. Normally the inorganic sulfate is present at about four times the organic levels. Exposure to benzene tends to increase the organic sulfate and lower the inorganic. Data from studies by Hammond and Herman (1960) suggest that of total sulfates, urinary inorganic sulfate levels of 80-95 percent were normal, 70-80 percent indicated some exposure to benzene, 60-70 percent suggested a dangerous level of benzene exposure, and 0-60 percent indicated that there had been benzene exposure sufficiently high to create an extremely hazardous situation.

In humans the sulfate is the major conjugate of phenol until levels of approximately 400 mg/l are reached (Sherwood, 1972). Beyond that level glucuronides are found. Teisinger, et al. (1952) exposed humans to benzene at 100 ppm for 5 hours and found that their urine contained primarily phenol with small amounts of catechol and hydroquinone. It would appear from the available evidence, that benzene metabolism in humans is similar to that in animals.

The metabolism of benzene has been reviewed recently by Rusch, et al. (1977).

Excretion

Following exposure to benzene, humans, like animals, eliminate unchanged benzene in the expired air (Sherwood and Carter, 1970; Hunter, 1968; Nomiyama and Nomiyama, 1974a,b; Sato, 1972; Srbova, et al. 1950). The elimination of unchanged benzene was quantified in a series of studies by Nomiyama and Nomiyama (1974a,b) who exposed men and women to benzene at levels of 52-62 ppm for four hours and determined its respiratory disposition. A mean value of 46.9 percent of the benzene was taken up in these subjects, 30.2 percent was retained and the remaining 16.8 percent was excreted as unchanged benzene in the expired air. Pharmacokinetic plots of respiratory elimination were interpreted to indicate that there were three phases to the excretion that could be described by three rate constants. There were no significant differences between men and women in these studies. Hunter (1968) exposed humans to benzene at 100 ppm and detected benzene in expired air 24 hours later and suggested that it was possible to back-extrapolate to the concentration of benzene in the inspired air.

Benzene toxicity in humans is usually caused by inhalation of ambient air containing benzene vapor. Following cessation of exposure the body burden of benzene is reduced either by exhaling benzene in the expired air or by metabolism. The exhalation of unchanged benzene has been studied in dogs (Schrenk, et al. 1941), rabbits (Parke and Williams, 1953), mice (Andrews, et al. 1977) and rats (Rickert, et al. 1979). Schrenk, et al. (1941) exposed dogs to 800 ppm benzene by inhalation and determined that the time necessary to rid the body of benzene was related to the duration of exposure because of the tendency of benzene to accumulate in body fat. Parke and Williams (1953) administered ^{14}C-benzene orally and recovered approximately 43 percent of the administered dose as unmetabolized benzene in trapped exhaled air. Andrews, et al. (1977) administered benzene to mice subcutaneously and recovered 72 percent of the dose in the air. Simultaneous treatment with both benzene and toluene (Andrews, et al. 1977; Sato and Nakajima, 1979b) or benzene and piperonyl butoxide (Timbrell and Mitchell, 1977) increases the excretion of unchanged benzene in the breath. These compounds appear to act by inhibition of benzene metabolism which thereby leaves more benzene available for excretion through the lungs.

Rickert, et al. (1979) reported that the excretion of unchanged benzene from the lungs of rats followed a biphasic pattern suggesting a two-compartment model for distribution and a $t_{1/2}$ of 0.7 hr. This agreed with experimental $t_{1/2}$ values for various tissues which ranged from 0.4 to 1.6 hr.

EFFECTS

Acute, Subacute and Chronic Toxicity

In man, acute benzene poisoning is characterized by nausea, vomiting, ataxia, and excitement, followed by depression and coma. Death is usually the result of respiratory or cardiac failure (Holvey, 1972). Benzene exposure causes acute toxic effects on the central nervous system. These have been reviewed by Gerarde (1960) and Browning (1965). Single exposures to benzene in the air at a concentration of 20,000 ppm have proved to be fatal within 5 to 10 minutes. Effects included headache, nausea, staggering gait, paralysis, convulsions, and eventual unconsciousness and death, usually following cardiovascular collapse. Giddiness and euphoria have also been reported. Severe nonfatal cases have exhibited similar symptoms, but recovered after a period of unconsciousness. Autopsy findings have indicated respiratory tract inflammation, lung hemorrhages, kidney congestion, and cerebral edema (Winek and Collom, 1971).

It has also been suggested that accidentally ingested benzene may have resulted in ulceration of the gastrointestinal mucosa (Appuhn and Goldeck, 1957; Caprotti, et al. 1962).

The chronic effects of benzene have recently been thoroughly reviewed by the National Academy of Sciences (1976) and the U.S. EPA (1977). These reports have served as the main source of data for this section and the sections on mutagenicity and carcinogenicity.

Benzene is a proven hematotoxin. In man it is causally related to pancytopenia and to acute myeloblastic leukemia. Pancytopenia refers to a decrease in all of the major circulating formed elements in the blood: erythrocytes (red blood cells), leukocytes (white blood cells), and thrombocytes (platelets). In mild cases of benzene hematotoxicity a decrease in only one of the circulating formed elements may be observed; e.g., anemia, leukopenia, thrombocytopenia. The term aplastic anemia denotes a relatively severe pancytopenia, usually associated with a marked diminution in bone marrow cellularity.

Acute myeloblastic leukemia, also referred to as acute myelogenous leukemia, is the type of acute leukemia most commonly observed in adults. In addition to pancytopenia and acute myeloblastic leukemia, which can be clearly causally related to benzene exposure in man, there are a number of other hematological disorders for which the observed association with benzene exposure is not of sufficient strength to prove causality. These disorders include chronic myelogenous leukemia and various lymphoproliferative disorders.

The following discussion will review the evidence linking benzene exposure with hematotoxicity in man. The focus will be on those few studies for which dose-response data are available. Other aspects to be covered include discussion of the potential mechanism of toxicity and review of the literature concerning possible variability in individual susceptibility to benzene. More extensive discussion of benzene hematotoxicity in man is presented in a number of recent reviews (U.S. EPA, 1978a,b; Goldstein and Laskin, 1977; Goldstein, 1977b; NAS, 1975, 1976; NIOSH, 1974; Snyder and Kocsis, 1975).

Evidence of a pancytopenic effect of benzene was first noted in 1897 by Santesson, who reported four cases of fatal aplastic anemia occurring in workers fabricating bicycle tires. Since then numerous case reports and surveys of occupationally exposed groups of workers have documented this association, and many reviews of these cases have appeared (International Labour Office, 1968; Bowditch and Elkins, 1939; Browning, 1965; Goldstein, 1977b; Hamilton, 1931; NAS, 1975, 1976; Hunter, 1944; NIOSH, 1974; Selling and Osgood, 1935; Snyder, et al. 1978; Snyder and Kocsis, 1975). The causal relationship of benzene to pancytopenia in man is most clearly supported by studies of groups of workers in whom the appearance of pancytopenia was temporally related to the inception of benzene use and in which the outbreak of hematological effects was ended by replacement of benzene with another solvent.

Systematic studies of the pancytopenic effects of occupational exposure to benzene were performed by Greenburg, et al. (1939), Goldwater (1941), and Goldwater and Tewksbury (1941). These investigators evaluated workers in the printing industry who had been exposed to benzene for 3 to 5 years after the introduction of a new industrial process. Air sampling revealed benzene concentrations ranging from 11 ppm to 1,060 ppm (median 132 ppm). The most frequent hematological abnormalities found in the 332 exposed individuals, as compared to 81 nonexposed controls, were anemia, macrocytosis, and thrombocytopenia. Of note is that an absolute lymphocytopenia was more common than was neutropenia. Hematological abnormalities were observed in 65 workers, 23 of whom were considered to be severely affected, six seriously enough to require hospitalization. Recovery from hematological disorders was demonstrated following replacement of benzene with other solvents (Goldwater and Tewksbury, 1941).

Other relatively large scale early studies of occupationally-exposed individuals include Wilson's (1942) study of 1,104 workers in an American rubber factory during World War II. Mild hematotoxicity was noted in 83 workers. Severe pancytopenia was seen in 25 workers; of these, nine were hospitalized and three died. Ambient benzene levels in the factory were reported to have averaged at about 100 ppm with peaks of 500 ppm. Helmer

(1977) reported evidence of hematological abnormalities in 60 of 184 workers in a rubber raincoat factory. Levels of benzene in factory air were reported to range from 137 to 218 ppm and were speculated to have been higher in the period before the sampling was done. Re-evaluation of the workers 16 months after cessation of benzene use revealed that 46 had recovered, but twelve still had significant effects, and two had died.

Pagnotto, et al. (1961) reported atmospheric benzene levels of up to 125 ppm, but with the majority of levels lower than 25 ppm, in a study of a rubber coating facility. In one plant of this facility evidence of benzene hematoxicity was present in five of 32 workers while in two other plants none of the six and one of nine workers, respectively, were affected. However, the latter individual had hematological effects serious enough to require hospitalization. This is somewhat reminiscent of an earlier study by Hutchings, et al. (1947) who studied an Australian Air Force workshop after discovery of a fatal case of aplastic anemia. Hutchings, et al. (1947) measured peak benzene concentrations well above 100 ppm in most areas of the workshop with occasional levels as high as 1,400 ppm; average benzene levels were in the range of 10 to 35 ppm. Comparison of 87 benzene-exposed individuals with 500 workers exposed to other hydrocarbons and 300 unexposed controls demonstrated that they shared only a slight tendency toward cytopenic effects. These observations of Pagnotto, et al. (1961) and Hutchings, et al. (1947) suggest the possibility of individual susceptibility to the pancytopenic effects of benzene.

Detailed descriptions of many cases of benzene-induced pancytopenia in industrially-exposed individuals have been reported from Italy, particularly by Vigliani, Saita, Forni, and their colleagues (Forni and Moreo, 1967, 1969; Forni and Vigliani, 1974; Forni, et al. 1971a,b; Saita, 1945; Saita and Dompe, 1947; Saita and Moreo, 1959, 1961, 1966; Saita and Sbertoli, 1962; Saita and Vigliani, 1962; Saita, et al. 1964; Vigliani, 1975; Vigliani and Forni, 1966, 1969, 1976; Vigliani and Saita, 1943, 1964). These studies reported benzene concentrations ranging from 20 ppm to 150 ppm, with many levels over 200 ppm in several factories.

In a series of papers, Aksoy and his colleagues collected a large amount of data relating occurrence of aplastic anemia to the use of benzene-containing adhesives in the shoemaking industry. This incidence was shown to have dramatically declined following replacement of the adhesive with a benzene-free substance. Benzene levels in air to which the workers were exposed were in the range of 150 to 650 ppm (Aksoy, 1977; Aksoy and Erdem, 1969, 1978; Aksoy, et al. 1966, 1971, 1972a,b, 1974a,b,c, 1975a,b, 1976a,b; Erdogan and Aksoy, 1973).

Effects in occupationally-exposed groups at relatively low benzene levels have been reported by Eastern European researchers. Doskin (1971) reported findings in 365 workers employed in a new chemical factory which could be interpreted as indicating that exposure from 10 ppm to 40 ppm benzene for less than one year produces mild hematological effects. Mild thrombocytopenia was the most common abnormality and mild anemia was also seen. These were observed in about 40 percent of the workers, usually in the first year of exposure. Doskin (1971) also reported lymphocytosis, a biphasic leukocyte response, and bone marrow hypercellularity in the exposed individuals. These effects have not been reported by other researchers. The actual benzene levels and monitoring procedures used in this study were not clearly defined. Smolik, et al. (1973) reported a decrease in mean serum complement level in 34 benzene-exposed workers when compared to a control group. Benzene levels to which the workers were exposed ranged from 3.4 ppm to 6.8 ppm and the duration of exposure was from 3 months to 18 years. A related study reported findings of decreased serum immunoglobulin levels and increased levels of leukocyte agglutinins in workers exposed to benzene and alkyl benzenes (Lange, et al. 1973a,b). Altered immune function as a result of benzene exposure has been reported in animals and in man (Revnova, 1962; Roth, 1972) and is conceivably related to the known effects of benzene on lymphocytes. A mechanism for the decrease in complement levels reported in association with benzene exposure by Smolik, et al. (1973) has not been elucidated. Khan and Muzyka (1970, 1973) noted an increase in red cell deltaaminolevulinic acid in 16 of 27 workers exposed to benzene. Four of the 16 affected individuals were reported to have been exposed to only 1.6 ppm benzene. The other 12 had reported earlier exposures from 6.4 to 15.6 ppm and more recent exposures to 1.6 ppm benzene. Studies performed utilizing rabbit reticulocytes provided some support for the authors' hypothesis that benzene may alter porphyrin metabolism (Wildman, et al. 1976). This finding has not been confirmed in man. Chang (1972) reported hematological abnormalities, particularly anemia and leukopenia, in 28 of 119 workers exposed to benzene in Korea. The author performed a detailed extrapolation of his findings resulting in derivation of an exponential function describing the benzene concentration and duration of exposure required for hematotoxicity:

$$y = (82.5)\ (0.77^{0.2x}) + 10.1$$

where y equals benzene concentration in ppm and x is work duration in months. No hematological toxicity was observed in 18 subjects exposed to 10 to 20 ppm benzene. The work population and exposures were incompletely characterized, leading to difficulty in interpreting the general relevance of these findings. Hematologic effects in workers exposed to similar levels of benzene have been noted by Girard, et al. (1970a,b). Girard and colleagues (Girard, et al. 1966, 1967, 1968, 1970a,b,c, 1971a,b; Girard and Revol, 1970), in a series of studies of human benzene hematotoxicity, noted frequent decreases in leukocyte alkaline phosphatase activity among 319 workers exposed to 10-25 ppm benzene. The clinical significance of these findings is unclear.

Interpretation of these studies has been difficult, particularly with regard to dose-response relationships. A major problem has been the almost universal presence of other solvents along with benzene in the occupational environment. It has been widely suggested that benzene may not be unique among common solvents in its ability to produce hematotoxicity. Reports in the older literature, however, which reported hematopoietic effects of toluene and xylene, almost certainly reflect solvent contamination with benzene. The other aromatic solvents, although not directly hematotoxic, are suspected to interact with benzene, perhaps by altering its metabolism and thereby affecting its toxicity.

Another major problem with the interpretation of existing studies involves the estimation of the dose to the individual, which may vary due to differences in work habits. This is a particular problem when considering low incidence phenomena such as benzene leukemogenesis. A further problem in defining low level benzene effects is the wide range in the normal levels of blood formed elements (e.g., normal white blood cell count 5,000 - 10,000/mm^3; red blood cell count, 4.4 - 5.6 x 10^6/mm^3; platelet count 150,000 - 350,000/mm^3). Furthermore, the bone marrow has a considerable reserve capacity. Accordingly, the earliest hematopoietic effects of benzene may not be apparent when routine blood counts are obtained in an exposed population. Slight changes in hematological measurements which may be detected upon routine examinations of exposed individuals also may, in fact, be normal fluctuations. It is unknown whether minor shifts in hematological parameters should be considered as clinically insignificant or, on the other hand, could conceivably be the basis for neoplastic transformation or other hematotoxic manifestations.

The manifestations of benzene hematotoxicity range from clinically inapparent cytopenia to lethal aplastic anemia. Symptoms in milder cases appear to reflect anemia and include relatively nonspecific complaints such as lassitude, easy fatigability, dizziness, headache, palpitation, and shortness of breath. The direct life-threatening consequences of severe pancytopenia are from leukopenia, which results in decreased ability to fight infection, and from thrombocytopenia, which may precipitate significant bleeding. There is evidence suggesting that such effects are due not only to the absolute decrease in number, but also to qualitative abnormalities of circulating formed elements. Various alterations in morphology and function of granulocytes, lymphocytes, platelets, and red cells have been reported in humans exposed to benzene (U.S. EPA, 1978b). Some of these effects occur as relatively early manifestations of hematotoxicity and may be suitable for use as screening tests in populations with a history of exposure to benzene. Certain manifestations, e.g., macrocytosis, appear to be more frequent in benzene-induced pancytopenia than in cases of aplastic anemia of unknown etiology although there are no absolute data which prove or refute this hypothesis.

In terms of prognosis, review of the case material in the literature concerning benzene hematotoxicity suggests that the eventual outcome is similar to that reported for idiopathic aplastic anemia (Goldstein, 1977c). Mild cases tend to do well, with gradual recovery generally observed. On the other hand, severe aplastic anemia has a very high mortality rate even with modern therapeutic approaches. Moderate to severe cases may result in persistent pancytopenia. A particularly dreaded complication is the development of acute myeloblastic leukemia. This has occurred many years after cessation of benzene exposure (DeGowin, 1963; Erdogon and Aksoy, 1973; Guasch, et al. 1959; Justin-Besancon, et al. 1959; Saita and Vigliani, 1962; Sellyei and Kelemen, 1971).

There are three lines of evidence which support a causal relation between benzene exposure and acute myeloblastic leukemia in man. These include the basic biomedical data which produce a plausible conceptual framework for benzene leukemogenesis; the many case reports, including those in which individuals with almost certain benzene-induced pancytopenia have developed acute myeloblastic leukemia; and the epidemiological evidence obtained by different approaches, in different occupational settings, and in different countries, associating benzene with acute myeloblastic leukemia.

The correlation of benzene exposure with leukemogenesis has been compared with the well-known association of acute myeloblastic leukemia following idiopathic aplastic anemia resulting from other hematotoxins such as chloromycetin (chloramphenicol) or phenylbutazone. There is little difference in the clinical course of aplastic anemia following benzene exposure and exposure to other hematologically-active substances. The fact that benzene-induced pancytopenia is associated with chromosomal abnormalities in man is also in keeping with a causal relationship to neoplasia, although it does not constitute absolute proof thereof. Historically, benzene-induced leukemogenesis has not been demonstrated in laboratory animals. However, recent studies suggest low incidence of acute and chronic myelogenous leukemia in benzene-exposed animals of two rodent strains in which these diseases are not known to occur spontaneously (Goldstein, et al. 1980). Maltoni and Scarnato (1979) have previously demonstrated an enhancement of leukemia incidence in Sprague-Dawley rats when treated with benzene by ingestion in olive oil at concentrations of 250 and 50 mg/kg body weight once daily, 4-5 days weekly, for 52 weeks. In addition, these investigators observed high incidence of various types of malignancies, namely Zymbal's gland carcinomas, skin carcinomas, mammary carcinomas, angiosarcomas, hepatomas and tumors of other organs (Tables 4 and 5).

The world literature contains well over 100 case reports of acute myeloblastic leukemia in benzene-exposed individuals. There are reasonable grounds to expect that many cases may go unreported, as the association may be obscured by the often long delay between benzene exposure and manifestation of acute leukemia, or by ignorance of such exposure on the part of patient or physician. However, these case reports taken together do provide evidence of benzene casuality in three ways. The first is the worldwide distribution of the observations which have been reported in many different occupational settings and which have a single common denominator, benzene exposure. More impressive is the relative frequency in which individuals demonstrated to have benzene-induced pancytopenia have been followed clinically through a transitional preleukemic phase and then into acute myeloblastic leukemia. In these cases the relationship between the resulting leukemia and the initial benzene exposure can generally be clearly demonstrated. Also of note is the relative frequency of erythroleukemia, also known as Di Guglielmo's syndrome, among the case reports. This is a relatively uncommon variant of acute myeloblastic leukemia characterized by large numbers of circulating neoplastic red blood cell precursors. The apparent greater frequency of erythroleukemia in benzene-exposed individuals suggests a relatively specific effect of benzene on erythroid precursors which may differ from its effect on myeloid cells.

TABLE 4

Distribution of the Different Types of Tumors* +

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GROUPS No.	CONCENTRATION	ANIMALS (Sprague-Dawley rats, 13 weeks old at start)			ANIMALS WITH TUMOURS																				
					Zymbal gland carcinomas			Skin carcinomas			Mammary carcinomas			Leukemias			Angiosarcomas			Hepatomas			Others		
		Sex	No. at start	Corrected number (a)	Total No.	% (b)	Average latency time (weeks) (c)	Total No.	% (b)	Average latency time (weeks) (c)	Total No.	% (b)	Average latency time (weeks) (d)	Total No.	% (b)	Average latency time (weeks) (c)	Total No.	% (b)	Average latency time (weeks) (c)	Total No.	% (b)	Average latency time (weeks) (c)	Total No.	Benign No.	Malignant No.
I	250 mg/kg	♂	35	33	0	-	-	0	-	-	0	-	-	4	12.1	75.7	1 (f)	3.0	66.0	1	3.0	98.0	12	10	2
		♀	35	32	8	25.0	75.7	2	6.2	74.0	7	21.9	82.8	1	3.1	116.0	0	-	-	0	-	-	16	11	3
		♂ and ♀	70	65	8	12.3	75.7	2	3.1	74.0	7	10.8	82.8	5	7.7	82.6	1	1.5	66.0	1	1.5	98.0	28	21	5
II	50 mg/kg	♂	30	28	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	9	8	1
		♀	30	30	2 (e)	6.7	62.0	0	-	-	4	13.3	88.0	2	6.7	111.5	0	-	-	0	-	-	27	25	2
		♂ and ♀	60	58	2	3.4	62.0	0	-	-	4	6.9	88.0	2	3.4	111.5	0	-	-	0	-	-	36	33	3
III	Olive oil (Control)	♂	30	28	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	6	5	1
		♀	30	30	0	-	-	0	-	-	3	10.0	110.0	1	3.3	80.0	0	-	-	0	-	-	24	22	2
		♂ and ♀	60	58	0	-	-	0	-	-	3	5.2	110.0	1	1.7	80.0	0	-	-	0	-	-	30	27	3
Total			190	181																					

(a) Alive animals after 20 weeks, when the first tumour (a mammary fibroadenoma) was observed.
(b) The percentages are referred to the corrected number.
(c) Average time from the start of the experiment to the detection (at the periodic control or at autopsy).
(d) Average age at the onset of the first mammary tumour per animal detected at the periodic control or autopsy.
(e) 1 with sarcomatous component.
(f) Subcutaneous angiosarcoma.

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*Source: Maltoni and Scarnato, 1979.
+Exposure by ingestion (stomach tube) to benzene in olive oil at 250 and 50 mg/kg body weight, once daily, 4-5 days weekly, for 52 weeks. Results after 144 weeks (end of experiment).

TABLE 5

Distribution of the Different Types of Miscellaneous Tumors*a

GROUPS NO.	SEX	ANIMALS BEARING OTHER TUMOURS				Total
		Benign		Malignant		
		No.	Distribution of histotypes	No.	Distribution of histotypes	
I	M	10	4 dermatofibromas 2 subcutaneous lipomas 2 mammary fibromas 1 pheochromocytoma 1 lymph node fibroangioma	2	1 intrabdominal adenocarcinoma 1 oligodendroglioma	12
	F	13	9 mammary fibroadenomas 1 kidneys adenomal 1 pheochromocytoma 1 polypoid adenoma of the colon 1 polypus of the uterus	3	1 adenocarcinoma of the uterus 1 fibrosarcoma of the uters 1 meningioma	16
II	M	8	5 mammary fibromas 1 mammary fibroadenoma 2 Leydig cells tumours	1	1 meningioma	9
	F	25	21 mammary fibroadenomas 1 pheochromocytoma 1 polypus of the uterus 1 papilloma of the uterus 1 peritoneal lipoma	2	1 carcinoma of the ureter 1 adenocarcinoma of the uterus	27
III	M	5	2 dermatofibromas 1 pheochromocytoma 1 bladder papilloma 1 retroperitoneal lipoma	1	1 oligodendroglioma	6
	F	22	16 mammary fibroadenomas 1 adrenal gland cortical adenoma 1 pheochromocytoma 1 ileo-caecum fibroma 1 polypus of the uterus 1 bladder papilloma 1 neurilemoma	2	2 adenocarcinomas of the uterus	24

*Source: Maltoni and Scarnato, 1979.

[a] Exposure by ingestion (stomach tube) to benzene in olive oil at 250 and 50 mg/kg body weight, once daily, 4-5 days weekly, for 52 weeks. Results after 144 weeks (end of experiment).

The epidemiologic evidence supporting the relationship of benzene exposure to leukemia has been obtained using a number of different approaches. In some studies the starting point has been the observation by hematologists or by occupational physicians of individuals with leukemia. A case control approach in which occupational histories are obtained from individuals with various hematological disorders, as well as from normal subjects, has been used by French investigators who noted a significant association of past benzene exposure in patients with acute myeloblastic leukemia (Girard and Revol, 1970; Girard, et al. 1968). A recent study by Mitelman, et al. (1979) suggested that it may be possible to define a subgroup of leukemics with benzene-induced myeloblastic leukemia. Mitelman, et al. (1979) found chromosomal abnormalities in leukemic bone marrow cells in each of 13 individuals with an occupational history of solvent exposure, in each of three individuals who had been exposed to insecticides, and in three of seven exposed to petroleum products. In comparison, only eight of 33 leukemics with no history of such occupational exposure had chromosomal abnormalities. These findings suggest a specific mode of action in benzene leukemogenesis. Another epidemiologic approach has been taken by Italian and Turkish investigators who have evaluated large numbers of individuals with benzene associated hematotoxicity. Starting with well-defined cases, the investigators have estimated the total population exposed to benzene and then calculated the excess risk based upon the incidence of leukemia in the general population. Vigliani and Saita (1964) estimated a 20-fold increase in risk for acute leukemia in benzene-exposed workers in Pavia and Milan. Aksoy, et al. (1974b) reported a greater than 2-fold increase in risk for shoe workers in Istanbul, but, as discussed elsewhere (U.S. EPA, 1978b) this is probably an underestimate.

The episode in Turkey is particularly illustrative of the hematotoxic hazard of benzene. Beginning about 1960, shoemakers in Istanbul started using adhesives prepared with benzene (9 to 88 percent) which were cheaper than their customary adhesives. Aplastic anemia was first noted in this population in 1961 and acute myeloblastic leukemia in 1967. A series of reports by Aksoy and his colleagues have thoroughly described these findings (Aksoy, 1977; Aksoy and Erdem, 1969, 1978; Aksoy, et al. 1966, 1971, 1972a,b, 1974a,b,c, 1975a,b, 1976a,b). Of particular note is the obvious temporal relation to the onset of benzene use and the decline in new cases since replacement of the benzene-containing adhesive in 1969. In a recent review of 44 pancytopenia patients, Aksoy and Erdem (1978) noted that six had developed leukemia. Previously they had observed 26 shoemakers with acute leukemia during the period from 1967 to 1973. Also of interest is that the peak incidence of acute leukemia appeared to follow that of aplastic anemia by a few years. This is in keeping with the delayed onset of acute leukemia frequently noted in case reports.

Another standard epidemiologic approach is the retrospective study, which involves selection of a well-characterized population at risk and establishment of mortality patterns for that group. A number of major studies of this type have been performed in the rubber industry and among other workers exposed to solvents. Increased incidences of cancers of the lymphatic and hematopoietic systems were noted in male rubber workers when compared to all manufacturing industry workers who died in 1959 (U.S. Dept. of HEW, 1961). A higher incidence in deaths from these causes, particularly from lymphocytic leukemia, was also observed in a series of studies evaluating the ten year mortality experience of male workers at four tire manufacturing plants (Andjelkovic, et al. 1976, 1977; McMichael, et al. 1974, 1975, 1976a,b). Some tendency toward an increase in hematopoietic neoplasms was noted by Monson and Nakano (1976a,b) in their studies of mortality in rubber industry workers. However, no apparent increase in hematopoietic neoplasms was noted by Mancuso and his colleagues in studies on occupational cancer which focused on the rubber industry (Mancuso, et al. 1968; Mancuso and Brennan, 1970). Infante, et al. (1977a,b) have recently reported a study of the mortality of workers exposed to benzene in the rubber industry. Among 140 deaths observed in a cohort of 718 white males, seven leukemia deaths were observed as opposed to fewer than 2 expected. Infante, et al. (1977a,b) contended that benzene levels to which the affected individuals were exposed did not exceed the Threshold Limit Value (TLV). This has been questioned in view of testimony before the Occupational Safety and Health Administration (OSHA) that levels exceeded 220 ppm in certain plant areas (Harris, 1977).

Another recent mortality study by Ott, et al. (1978) found a higher incidence of acute myeloblastic leukemia than expected among 594 chemical workers with a history of benzene exposure. Of note is the observation by U.S. EPA (1978a) that dose extrapolations derived from the findings of Ott, et al. (1978) are not incompatible with those derived from Infante, et al. (1977a,b) when inherent uncertainties in dose extrapolation are taken into consideration.

Other epidemiological information which may be pertinent to benzene leukemogenesis include the observation of higher than expected mortality rates due to tumors of the lymphatic and hematopoietic systems among members of the American Chemical Society (Li, 1969). Similarly, the highest leukemia rates among British males divided into 27 occupational groups were found in the group described as "professional, technical workers, artists"

(Adelstein, 1972). This would include many types of workers exposed to benzene as well as to radiation. A possible interaction between benzene exposure and radiation was observed in a retrospective case control study of Japanese atom bomb survivors in which those who developed leukemia had significantly greater benzene and x-ray exposures than those who did not develop leukemia (Ishimaru, et al. 1971). A study of cancer mortality among employees of the U.S. Government Printing Office reported an increased proportionate mortality ratio for leukemia among binding workers who may have been exposed to benzene (Greene, et al. 1979).

Other epidemiologic studies which have not found an association between benzene exposure and hematologic neoplasms include a large-scale investigation of petroleum workers in which there was not an increased incidence of acute leukemia (Thorpe, 1974). Furthermore, no statistically significant increase in leukemia mortality has been observed in coke plant workers who are exposed to benzene (Redmond, et al. 1972, 1976).

A more detailed critique of these studies is provided in the previous EPA review of benzene exposure (U.S. EPA, 1978b). Inherent in epidemiologic investigations is the inability of any one study to prove causality. However, taken as a group, these studies of defined work populations leave little doubt that benzene is leukemogenic in man.

In addition to pancytopenia and acute myeloblastic leukemia and its variants, benzene has been implicated as causally related to a number of other hematologic disorders. These include myeloproliferative disorders such as chronic myelocytic leukemia, myeloid metaplasia, and essential thrombocythemia, and lymphoproliferative disorders such as acute and chronic lymphocytic leukemia, lymphocytic lymphomas, and a paraneoplastic condition known as paroxysmal nocturnal hemoglobinuria. Case reports of benzene-exposed individuals who developed these disorders have been compiled by Goldstein (1977b), and the strength of the association has been discussed by the U.S. EPA (1978a). The causal relation between benzene and any one of these diagnostic categories remains unproven. There is, however, reasonably good evidence that a relationship does exist for chronic myelocytic leukemia, myeloid metaplasia, chronic lymphocytic leukemia, and paroxysmal nocturnal hemoglobinuria and benzene exposure. Epidemiologic evidence of the association of benzene exposure with lymphoma has been recently described by Vianna and Polan (1979) who reported an excess of deaths due to lymphoma in males who had worked in various occupations where there was exposure to benzene and/or coal tar fractions. The data are confounded by the preponderance of farmworkers in the study cohort; the extent of exposure of this occupational subgroup is not known.

Individual host factors may account for variation in the degree of susceptibility to benzene. These include obesity, possibly because of the increased solubility of benzene in fat; age, with younger individuals perhaps at greater risk; sex, with females suggested to have greater risk; and high ambient temperatures (Doskin, 1971; Greenberg, et al. 1939; Ito, 1962; Mallory, et al. 1939). However, the evidence for any of these risk factors is less than compelling. There is also some evidence of a familial tendency to benzene hematotoxicity, suggesting genetic predisposition, but this is also unproven (Aksoy, et al. 1974a; Erf and Rhoads, 1939). Similarly, observations suggesting that individuals with increased bone marrow turnover times are more at risk for benzene hematotoxicity, while plausible, require experimental confirmation (Aksoy, et al. 1975a; Gaultier, et al. 1970; Saita and Moreo, 1959). Individual differences in rates of benzene metabolism would also be expected to affect toxicity. In addition to genetic factors, the ingestion of food, alcohol, or drugs, or the inhalation of other solvents, might alter benzene metabolism.

The possibility of individual variation in response should be considered as a possible explanation for the range of effects manifested as benzene hematotoxicity. Pancytopenia has been, however, practically ruled out as an idiosyncratic response to benzene. Based on animal studies, and on evaluations of occupationally exposed groups in which most individuals appear to have been effected, it seems clear that the pancytopenic effect of benzene exhibits classic dose-response characteristics. An idiosyncratic reaction is perhaps more likely to be an explanation for benzene leukemogenesis in view of the lower incidence of leukemia when compared to pancytopenia and related disorders when large occupational groups are studied. For instance, in a restudy of 125 of a group of 147 workers evaluated nine years previously and of whom 107 had abnormal blood counts, only one was reported to have developed acute leukemia (Goldstein, 1977b; NIOSH 1974). However, Aksoy and Erdem (1978) recently reported that 6 of 44 significantly pancytopenic individuals that they followed developed acute leukemia. Fourteen of the forty-four had died from aplastic anemia. This suggests that the propensity to develop acute leukemia as a result of benzene exposure may not be rare. Based on present information, it is not unreasonable to assume that everyone is at risk for benzene leukemogenesis.

In its production of hematotoxicity, benzene is relatively unique among solvents. Most related compounds have negligible effects, if any, on the bone marrow. Little is known concerning the mechanism by which benzene exposure leads to hematotoxicity. The evidence suggests that it is a metabolite of benzene which is toxic to hematopoietic precursors. The identity of this metabolite is unknown as is its physiochemical mode of action. Of particular interest would be information as to whether the metabolite(s) is (are) responsible only for destruction of precursor cells leading to pancytopenia or is (are) also capable of producing somatic mutation leading to leukemia.

Chronic effects of benzene on the immunological system have been reported. Lange, et al. (1973a) found decreased levels of IgG and IgA and increased levels of IgM in workers exposed to a combination of benzene, toluene, and xylene. Lange, et al. (1973a) reported that the following air concentrations of benzene had been measured in the work atmosphere: 0.11-0.158 mg/l benzene, 0.203-0.27 mg/l toluene, and 0.224-0.326 mg/l xylene for samples taken early in the study, and 0.0122-0.022 mg/l benzene, 0.08-0.23 mg/l toluene, and 0.12-0.63 mg/l xylene for samples taken at a later date. Some of these workers were found to have autoleukocyte agglutinins, suggesting the occurrence of an allergic blood dycrasia in some people exposed to benzene and its homologs (Lange, et al. 1973b). Smolik, et al. (1973) have found significantly lower serum complement levels in workers exposed to benzene, toluene, and xylene.

Although the causal relationship between benzene exposure and human disorders is clear, the literature does not allow any conclusions to be drawn on the dose-response relationship between benzene and these disorders in humans. Some dose-response data on the effects of benzene on animals, however, exist. Wolf, et al. (1956) reported that the no-effect level for blood changes in rats, guinea pigs, and rabbits was below 88 ppm in the air when the animals were exposed for 7 hr/day for up to 269 days. At this level slight leukopenia was observed in rats; leukopenia was also seen in rats given 132 daily oral doses of 10 mg/kg for 187 days. Jenkins, et al.

(1970) found no effects on the blood composition of rats, guinea pigs, and dogs exposed continuously to 17.65 ppm benzene for up to 127 days. Slight leukopenia has been reported to occur in rats exposed to 44 ppm benzene for 5 hrs/day, 4 days/week for 5 to 7 weeks (Deichmann, et al. 1963). Deichmann, et al. (1963) exposed Sprague-Dawley rats to 15 to 831 ppm benzene vapor for 20 hours/week for 6 to 31 weeks. Rats exposed to a mean concentration of 65 ppm for 26 out of 39 days showed a decrease in white blood cell count after 2 weeks in males and after 4 weeks in female rats. Animals exposed to 47 ppm and 31 ppm exhibited abnormalities of the spleen and lungs. Rats exposed to 831 ppm for 32 of 46 days showed a decrease in white blood cell count that remained constant throughout the period of exposure.

Sprague-Dawley rats of different ages received oral doses of undiluted benzene to determine the oral LD_{50} (Kimura, et al. 1970). Acute LD_{50} reported are: immature rats, 3.4 g/kg; young adult rats, 3.8 g/kg; old adult rats, 5.6 g/kg. A concentration of 0.87 g/kg body weight proved fatal to newborn rats.

Dobashi (1974) measured the cell renewal rate as well as the rate of DNA synthesis in cultured human leukocytes and HeLa cells exposed to benzene. Both cell types exhibited 50 percent inhibition of growth at 171.6 mg/l. The rate of DNA synthesis in leukocytes was inhibited by 50 percent at 171.6 mg/l, while in HeLa cells this effect occurred at 85.8 mg/l benzene.

Synergism and/or Antagonism

The interaction of benzene with other solvents such as xylene and toluene alters the rate of metabolism of benzene, thereby affecting benzene toxicity. Animal investigations have indicated that benzene, toluene, and perhaps other aromatic solvents, are oxidized by many of the same hepatic enzyme systems (Ikeda, et al. 1972).

Most reports on the human health effects of benzene have originated in workers exposed to high concentrations of benzene in conjunction with other solvents, e.g., toluene and xylene. Thus, it has been suggested that benzene might act synergistically with other compounds to enhance hematotoxicity. This synergism might possible explain the failure to induce leukemia in animals with benzene (NAS, 1976).

Andrews, et al. (1977) suggested that benzene-induced bone marrow toxicity might be inhibited by co-administration of toluene due to inhibition of hydroxylation of benzene. Inhibition of benzene metabolism by toluene may result in increased toxic effects which may be due to benzene itself. The toxic effects of benzene on the bone marrow are suspected to result from action of metabolites of benzene.

Teratogenicity

The interest in the potential teratogenic effect of benzene is based on the current recognition that some organic solvents are known to produce congenital malformations in experimental animals. Furthermore, the reported pancytopenia seen in workers exposed to toxic levels of benzene has raised the possibility that benzene could adversely affect the cells of a developing embryo.

The first report of benzene-induced teratogenicity was by Watanabe and Yoshida (1970) who administered a very high dose of benzene subcutaneously (3 ml/kg body weight) to pregnant mice on day 13 of gestation. The fetuses that were delivered by caesarian section on day 19 showed anomalies such as cleft palate, agnathia (no lower jaw), and micrognathia (reduced lower jaw). Other externally visible defects did not appear. The authors also reported that no skeletal defects appeared in the vertebrae, ribs, or extremities. However, the anomalies produced have been shown to be commonly encountered in normal or nonexposed mice.

The noninhalation route of administering benzene has been used in two other studies. Recently, Nawrot and Staples (1979) reported that administration of benzene by gavage (0.3, 0.5, and 1.0 ml/kg) to CD-1 strain mice during days 6-15 of gestation resulted in significant maternal lethality and in embryonic resorption. However, these authors also stated that no significant benzene-related change in incidence of malformation was seen in animals given 1 mg/kg benzene during days 6-15 or during days 12-15 of gestation. There were no congenital malformations in offspring of male mice that were administered benzene intraperitoneally and subsequently mated to nonexposed females (Lyon, 1975).

The inhalation studies summarized in Table 6 show no congenital malformations in offspring of benzene-treated dams. Results varied from decreased fetal weight to reduced number of fetuses per litter, to no effects at all. Differences in animal strain, purity of compound, and duration of inhalation could possibly account for some differences in results.

Four additional inhalation studies are summarized in Table 7. These studies were designed to identify the effects of inhaled benzene vapor on fetal growth and development. Thus, the exposure was limited to the period of organogenesis, i.e., days 6 to 16 of gestation for rats and mice, and days 6 to 18 of gestation for rabbits. Inhalation chambers, generally of one cubic meter size, were employed, and animals were exposed to levels of benzene ranging from 10 to 2,200 ppm for 6 to 7 hours per day.

In most inhalation studies summarized in Tables 6 and 7, the exposure to benzene vapor affected the pregnant animal. Decreased gain in maternal body weight with concommitant retardation of fetal growth can be related to reduced food consumption during the treatment period, thus contributing to the physiologic and metabolic stress of high doses of benzene. Unfortunately, simultaneous analyses of benzene levels in maternal blood during the

TABLE 6

Benzene Teratology and Related Studies

STUDY	SPECIES	EXPOSURE LEVEL	ROUTE AND DURATION OF EXPOSURE	DECREASED MATERNAL WEIGHT GAIN	DECREASED FETAL WEIGHT GAIN	COMMENTS ON OBSERVATIONS
Watanabe a Yoshida (1970)	Mouse	3 ml/kg	Subcutaneous 11-15 days	None		Cleft palate, agnathia, micrognathia
Lyon (1975)	Rat	0.5 ml/kg	Intraperiotneal			No effect on Offspring. [Exposure of males in Dominant Lethal Study]
Nawrot a Staples (1979)	Mouse	0.3-1.0 ml/kg	Gavage			Embryonic Resorption
Gofmekler (1968)	Rat	210 mg/m^3 (65 ppm)	Inhalation 24 Hr./Day 10-15 days prior to mating			Decr. litter size
Puskina, et al. (1968)	Rat	670 mg/m^3 (208 ppm)	Inhalation throughout pregnancy			Decr. litter size
Vozovaya (1975)	Rat	1783 mg/m^3 (559 ppm)	Inhalation 4 mo. prior. plus throughout pregnancy		Yes - not sig.	No malformations for two generations

TABLE 6 (continued)

Benzene Teratology and Related Studies

STUDY	SPECIES	EXPOSURE LEVEL	ROUTE AND DURATION OF EXPOSURE	DECREASED MATERNAL WEIGHT GAIN	DECREASED FETAL WEIGHT GAIN	COMMENTS ON OBSERVATIONS
Vozovaya (1976)	Rat	370 mg/m^3 (116 ppm)	Inhalation 4 mo. prior, plus throughout pregnancy		Yes	No malformations
Hudak & Ungvary (1978)	Rat Mouse	1,000 mg/m^3 (310 ppm)	Inhalation 24 Hr./Day 1 to 14 days of pregnancy		Yes	No malformations

TABLE 7

Summary of Benzene Inhalation Terotology

STUDY	SPECIES	STRAIN	INHALATION EXPOSURE STUDY (ppm)	DURATION	DECREASED MATERNAL BODY WEIGHT	DECREASED FETAL BODY WEIGHT	DECREASED CROWN RUMP DISTANCE	COMMENTS OR OBSERVATIONS
Hazelton, 1975 (as cited in Murray, et al. 1979)	Rat	Sprague-Dawley	0 10 50 500	day 6 to day 16 of gestation	- - * *	- - * *	- - - *	- - - malformations(I)
Green, et al.1977	Rat	Sprague-Dawley	100 300 2,200	day 6 to day 16 of gestation	- - *	- - *	- - *	missing sternebra* missing sternebra* (most in females) missing sternebra* (most in females)
Murray, et al. 1979	Mouse	CF-1	500	day 6 to day 18 of gestation	-	*	-	missing sternebra*; delayed skull ossification*; unfused occipital*
	Rabbit	New Zealand	500	day 6 to day 18 of gestation	-	-	-	extra ribs*; lumbar spur(s)*

*Statistically significant (p<0.05)

(I)exencephaly, angulated ribs, out-of-sequence ossification of forefeet period of exposure are not available to provide data on the amount of circulating benzene or its metabolites accessible for possible absorption across the placental barrier.

Variations in number of sternebrae and ribs reported in several studies are not generally considered malformations in the absence of other anomalies (Kimmel and Wilson, 1973). An extensive report by Kimmel and Wilson (1973) of skeletal deviations in rats, concluded that skeletal variants of this type alone are not useful indicators of teratogenic potential. Palmer (1968) reported that extra ribs are also a common occurrence in New Zealand white rabbits. Incomplete ossification of the occipitals in the skull occurs in 10 to 11 percent of control fetuses of Sprague-Dawley strain (Charles River derived rats) according to Banerjee and Durloo (1973). Delayed ossification of sternebrae is indicative of growth retardation which may be attributed to nutritional imbalance.

The fetal malformations reported in the rat inhalation studies are summarized in Table 7. Three types of malformation were reported at an exposure level of 500 ppm in the Hazelton Study (Murray, et al. 1979). They include one exencephalic pup in 151 examined, one pup with angulated ribs in 107 pups examined, and two pups from two different litters with nonsequential ossification of the forefeet in 107 pups examined. Exencephaly may be induced by food deprivation for as little as 24 hours (Runner and Miller, 1956). Miller (1962) reported that 24 hours of fasting in mice altered vertebrate and rib formation. Thus, these malformations may have resulted from maternal nutritional stress, or in view of the low incidence, may have occurred entirely by chance. These findings were not reported in any other study and, despite exposure of 184 fetuses to 2,200 ppm benzene by Green, et al. (1978), no effects on the fetuses were observed.

In the Hazelton Study (Murray, et al. 1979), the negative control group contained only 12 pregnant females, fewer than in any of the treatment groups (16, 15, and 14 pregnant rats at 10, 50, and 500 ppm, respectively). The FDA guidelines published in 1966 specify that a minimum of 20 pregnant animals per group are necessary to provide statistically significant results. Since each group began with 20 females and insemination occurred prior to benzene exposure, such a low fertility index suggests the possibility of environmental or physical stress unrelated to the chemical. In addition, when fewer pups are available for evaluation in the control group than in any treatment group, the likelihood of a spontaneous malformation arising in the treatment group without a similar occurrence in the controls is increased and such malformation cannot absolutely be considered a teratogenic event.

Rat inhalation studies were performed by Green, et al. (1978) at exposure levels of 100, 300, or 2,200 ppm and in the Hazelton Study (Murray, et al. 1979) at 10, 50, or 500 ppm. There were no significant changes in incidence of resorptions at exposure levels as high as 2,200 ppm or 500 ppm, respectively (Table 8).

Although chronic exposure to benzene may constitute a fetotoxic or teratogenic hazard, the inhalation studies discussed are too inconclusive to either confirm or refute the hypothesis. Coincidentally, a recent review of the embryonic and teratogenic effects of benzene concluded with the following statement: "Since reports of effects of benzene on teratogenesis are few, and the concentrations of benzene used are very high, the role of benzene in teratogenesis cannot be predicted with confidence at this time" (U.S. EPA, 1978b).

In summary, from available data, it is unlikely that benzene administered by inhalation during the principal period of organogenesis constitutes a teratogenic hazard. However, results are not conclusive and do not apply

TABLE 8

Effect of Benzene Inhaled by Pregnant Rats on the Physical Parameters of the Litter

	GREEN, et al. 1978						MURRAY, et al. 1979 (HAZELTON STUDY)			
BENZENE CONCENTRATION (PPM)	CONTROL --	BENZENE 100	CONTROL --	BENZENE 300	CONTROL --	BENZENE 2,200	CONTROL --	BENZENE 10	BENZENE 50	BENZENE 500
Number of Litters	16	18	15	16	14	15	11	15	15	14
Implantation Sites/Litter	11.8	11.8	11.9	13.4	12.6	13.0	10.8	13.1	8.7	11.8
Live Fetuses/ Litter	10.4	11.8	11.0	12.2	12.1	12.3	9.7	12.5	8.5	10.8
Percent Resorptions/ Implantation Sites*	11 (22/188)	5 (12/255)	7 (14/179)	9 (20/125)	5 (8/151)	4 (8/195)	10.1 (12/119)	4.1 (8/197)	3.1 (4/131)	8.5 (14/165)
Percent Litters with Resorptions*	50 (8/16)	44 (8/18)	46 (7/15)	62 (10/16)	42 (6/14)	33 (5/15)	45 (5/11)	33 (5/15)	20 (3/15)	57 (8/14)
Litters Totally Resorbed	0	0	0	0	0	0	1	0	0	0
Resorptions/ Litter with Resorptions**	2.8 (22/8)	1.5 (12/8)	2.0 (14/7)	2.0 (20/10)	1.3 (8/6)	1.6 (8/5)	2.4 (12/5)	1.6 (8/5)	1.3 (4/3)	1.8 (14/8)

*(N_1/N_2) represents the number actually observed over the total number possible.

**(N_1/N_2) represents total number of resorptions per litter in which resorptions occurred.

to other stages of the reproductive cycle. The effects of benzene on male and female fertility, preimplanation development, parturition, and lactation need to be evaluated.

Mutagenicity

The cytologic and cytogenetic effects of benzene have been thoroughly reviewed by Wolman (1977), and in the 1978 EPA Review of benzene health effects. Benzene has not shown mutagenic activity in the *Salmonella*/microsome *in vitro* assay (Lyon, 1975; Shahin, 1977; Simmon, et al. 1977). It has shown such activity, however, in animals and man. Chromosomal abnormalities in bone marrow cells have been reported as a consequence of experimental benzene exposure in a number of species, including rats (Lyapkalo, 1973; Dobrokhotov, 1972; Philip and Jensen, 1970; Snyder, et al. 1978), rabbits (Kissling and Speck, 1972), mice (Snyder, et al. 1978), and amphibians (Rondanelli, et al. 1961, 1964). In rabbits injected subcutaneously with 0.2 mg/kg/day benzene, the frequency of bone marrow mitoses with chromosomal aberrations increased from 5.9 percent to 57.8 percent after an average of 18 weeks (Kissling and Speck, 1972). Dobrokhotov (1972) exposed rats to 0.2 g/kg/day benzene and 0.8 g/kg/day toluene individually and together, and found similar rates of chromosomal aberrations in the two chemicals given separately, and an additive effect when given together. Chromatid deletions in metaphase chromosomes of bone marrow cells have been found in rats given single doses of subcutaneous benzene at 2 ml/kg (Philip and Jensen, 1970), and rats given subcutaneous benzene at 1 g/kg/day for 12 days (Lyapkalo, 1973). Lyon (1975) dosed rats with 0.5 ml/kg benzene intraperitoneally, and found no induced dominant lethality but increased chromatid and chromosomal abberrations. Lyon (1975) also found increased micronuclei counts 6 hours after final dosing of rats at 0.05 and 0.25 ml/kg intraperitoneally on each of two successive days. Cytogenetic abnormalities have also been observed in human lymphocytes cultured in vitro in the presence of benzene. Koizumi, et al. (1974) observed gaps and breaks in chromosomes of human leukocytes incubated for 72 hours at 37°C in tissue culture medium containing 30 percent calf serum to which benzene in a concentration of 2.2×10^{-3}M, 1.1×10^{-3}M, 2.2×10^{-4}M, 1.1×10^{-4}M, or 2.2×10^{-5}M was added. Haberlandt and Mente (1971) also reported chromosomal aberrations in human leukocyte cultures treated with benzene.

Benzene is clearly a mitotic poison, producing a decrease in DNA synthesis in animal bone marrow cells in vitro and in cultured human cells (Boje, et al. 1970; Dobashi, 1974; Kissling and Speck, 1972; Koizumi, et al. 1974; Matsushita, 1966; Speck, et al. 1966). There is also ample evidence of cytogenetic abnormalities in benzene-exposed individuals particularly those with clinically evident hematotoxicity (Buday, et al. 1971; Cobo, et al. 1970; Erdogan and Aksoy, 1973; Forni and Moreo, 1967; Forni, et al. 1971a; Harberlandt and Mente, 1971; Hartwich, et al. 1969; Marchal, 1952; Pollini and Colombi, 1964; Pollini, et al. 1964). Such abnormalities may persist for many years despite cessation of benzene exposure (Forni, et al. 1971b). A more important, and still controversial, consideration has been whether or not occupational exposure to benzene levels not producing overt hematological effects are capable of causing chromosomal abnormalities.

In patients with benzene-induced aplastic anemia, lymphocyte chromosome damage, i.e., abnormal karyotype and deletion of chromosomal material, has been found (Pollini and Colombi, 1964). Pollini, et al. (1964) later found a 70 percent incidence of heteroploid chromosomal patterns in the blood lymphocytes and bone marrow parenchyma cells of each of four subjects with benzene-induced blood dyscrasia. Chromosomal alterations associated with benzene-induced blood dycrasias have also been reported by others (Forni and Moreo, 1967, 1969; Hartwich, et al. 1969; Khan and Khan, 1973; Sellyei and Kelemen, 1971; Tough and Court-Brown, 1965).

In a more recent study, Funes-Cravioto, et al. (1977) have reported a significantly increased frequency of chromatid and isochromatid breaks in the cultured lymphocytes of workers in chemical laboratories and in the printing industry. A total of 73 individuals from seven different occupational groups of 15 or fewer members each were evaluated. Exposure to benzene was suspected or documented in each group. In some cases there had been sufficiently high exposure to result in hematological effects. The authors discounted the likelihood of x-irradiation significantly contributing to the results. As with many of the other studies in occupational groups, there was a relatively low correlation between length of exposure and frequency of chromosome breaks. The authors also noted an increased frequency of sister chromatid exchange in the lymphocytes of 12 laboratory technicians but not in 4 rotoprinting workers as compared to control groups. Of particular note in this study is the finding of a significantly higher level of chromosome aberrations in the children of 14 mothers who had been exposed to solvents during pregnancy while working as laboratory technicians; chromosomal aberrations were found in 7 children of non-exposed mothers (9.8 percent abnormal cells vs. 2.4 percent abnormal cells, $p \leq .01$). In other studies which evaluated relatively healthy workers, chromosome changes were detected in workers who were exposed to less than 10 ppm benzene (Berlin, et al. 1977; Kilian and Daniel, 1978; Picciano, 1978).

Vigliani and Forni (1969) found a significant increase of peripheral blood lymphocyte chromosomal aberrations in workers exposed to benzene, but not in those exposed to toluene and xylene. Some of these aberrations persisted for several years after recovery from benzene hemopathy. The authors hypothesized that leukemia may develop in cases where a potentially leukemic clone of cells with selective advantage is produced as a response to benzene exposure. Forni, et al. (1971a) examined chromosomal aberrations in 34 workers in a rotogravure plant and 34 matched controls, and found a significantly higher number of both stable and unstable aberrations in the 10 benzene-exposed workers but a normal number in the 24 toluene-exposed workers. Forni, et al. (1971b) found significantly increased stable and unstable chromosomal aberrations in 25 subjects who had recovered from benzene hemopathy. Most of these persisted for several years after cessation of exposure and recovery. A correlation between benzene exposure and chromosomal aberrations has been reported by Tough, et al. (1970) and Hartwich and Schwanitz (1972), in the latter case after "low levels" of benzene exposure.

A recent report (Kilian and Daniel, 1978) on 52 workers exposed to benzene for 1 month to 26 years (mean of 56.6 months) found chromosomal aberrations (chromosome breaks, dicentric chromosomes, translocations, and exchange figures) in peripheral lymphocytes at two to three times the rates found in controls. In this study the 8-hour average time-weighted benzene exposure was 2 to 3 ppm, the average concentration determined by 15-minute sampling was 25 ppm, and the peak concentration was 50 ppm.

Taken together these studies clearly indicate a causal relation between benzene exposure and persistent chromosomal abnormalities. The implications of such observations to benzene-induced leukemia are reasonably convincing in view of the analogous findings in radiation leukemogenesis as well as a large body of evidence supporting the role of somatic mutation in carcinogenesis. More evidence is needed before the slight, but statistically significant, increases in cytogenetic abnormalities observed in occupationally exposed workers can be related to leukemogenesis and ascribed with certainty to relatively low levels of benzene. At present, there is no correlation between the degree or length of exposure, the clinical symptoms, and the extent of persistence of chromosomal aberrations (U.S. EPA, 1977).

Carcinogenicity

Lignac (1932) reported the occurrence of leukemia in 8 of 33 albino mice subcutaneously injected with 0.001 ml benzene in 0.1 ml olive oil weekly for 17 to 21 weeks. These results remain in question, however, since no controls were used (Int. Agency Res. Cancer, 1974). Kirschbaum and Strong (1942) found leukemia in 6/20 mice (30 percent) subcutaneously injected with 0.001 ml benzene in sesame oil weekly, compared with 29/212 (14 percent) in controls, the difference being not statistically significant. Amiel (1960) gave weekly subcutaneous injections of 0.001 ml benzene in 0.1 ml olive oil for life to AKR, DBA2, CeH, and C57BL6 mice, in groups of 30 males. No cancer was found in any mice of the DBA2, C3H, and C57BL6 strains. Eight of 30 treated AKR mice developed leukemia, as did 30/35 untreated AKR mice. Hiraki, et al. (1963) injected five female and five male mice with 0.1 ml of a 1 percent solution (0.087 mg) of benzene in olive oil each week. Two mice died in 8 weeks; the remaining eight mice were treated for 10 weeks. Of these, two males and three females developed subcutaneous sarcomas. Three of these tumors were transplantable into syngenetic mice. No controls were reported. Ward, et al. (1975) subcutaneously injected weanling male C57BL 6N mice twice weekly for 44 weeks and once weekly for the last 10 weeks, gradually increasing the dose from 450 mg/kg to 1.8 g/kg benzene. The mice were killed 104 weeks after the first injection, and no evidence of carcinogenic activity was found in either the benzene-treated or negative control mice. Butylnitrosourea induced leukemia, lymphomas, and/or intestinal neoplasms in almost all the positive controls.

In a preliminary report, Maltoni and Scarnato (1979) found Zymbal's gland carcinomas in 8 of 32 female Sprague-Dawley rats which received 250 mg/kg benzene in olive oil by gavage once daily 4 to 5 days per week for 52 weeks, and in 2 of 30 female Sprague-Dawley rats which received 50 mg/kg. No such tumors were found in olive oil controls. Maltoni and Scarnato (1979) also reported increased incidence of mammary carcinomas in female rats and of leukemias in both male and female rats that were similarly treated (Table 4).

Numerous studies on the effects of skin application of benzene to mice (many where benzene was the solvent control) have yielded negative results (Baldwin, et al. 1961; Burdette and Strong, 1941; Coombs and Croft, 1966; Kirschbaum and Strong, 1942; Laerum, 1973). Inhalation (Jenkins, et al. 1970; Wolf, et al. 1956) and oral (Wolf, et al. 1956) dosing likewise have yielded negative carcinogenic results. In a recent inhalation study, Snyder et al. (1980) observed on increased incidence of thymic lymphoma in C57BL mice by exposing to 300 ppm of benzene (Table 9). It should be noted that C57BL strain carries a virus which can result in high incidence of lymphoma following exposure to radiation, carcinogens, or immunosuppressive agent (Koplan, 1967; Igel, et al. 1969; Imamura, et al. 1973). In the same Insert experiment using AKR mice, a strain which also carries a virus that can spontaneously induce lymphoma (Kahn and Novak, 1973), Snyder, et al. (1980) could not find any change in the induction of lymphoma in this strain by benzene. Nelson (1977) has found leukemia in 2/40 CD-1 mice given lifetime exposures to 300 ppm benzene; one had chronic myelogenous leukemia and one had an acute, possibly myeloblastic leukemia. A third mouse died of myeloid metaplasia. Nelson also found chronic granulocytic leukemia in 1/40 Sprague-Dawley rats given lifetime exposures to 100 ppm benzene.

TABLE 9

Histological Evaluation of C57BL Mice Exposed to 300 ppm Benzene and of Air Sham*

Neoplasm Type	Incidence	
	Test	Control
1. Hematopoietic neoplasms	8/40	2/40
2. Bone marrow hyperplasia without evidence of hematopoietic neoplasm	13/32	0/38
3. Spleen hyperplasia without hematopoietic neoplasm	16/32	2/38

*Source: Snyder, et al. 1980.

Thus far, animal experiences do not support conclusively the view that benzene is leukemogenic. Ward, et al. (1975) suggested that benzene-induced leukemia in man may be a fairly rare event occurring only in highly sensitive individuals, because of genetic constitution, or because of synergistic action with other environmental agents. Another point suggested to explain the difference between man and animal models was a difference in metabolism of benzene (NAS, 1976).

Despite essentially negative animal data, the evidence that benzene is a leukemogen for man is convincing and has recently been reviewed by NAS (1976), NIOSH (1977), and U.S. EPA (1977).

Over 250 cases of leukemia in benzene exposed individuals have been reported in the literature since the original description by Delore and Borgomano in 1928, essentially all of them in an occupational setting (Benzene in the Work Environment, 1974). These case reports, however, do not establish benzene as a leukemogen because of the possiblity of chance association, lack of information on the size of the population at risk, and chance of underreporting of benzene-associated leukemia due to acceptance of the relationship (thus inhibiting publication) or due to the lag period between exposure and onset of leukemia. These case reports do, however, suggest such leukemogenic properties. Conspicuous in these reports is the frequent description of persons suffering from benzene-associated pancytopenia in whom evolution to acute leukemia was observed. Idiopathic aplastic anemia is an uncommon disorder, reported far less frequently than acute myelogeneous leukemia. The relatively frequent documentation of benzene associated pancytopenia progressing to acute leukemia, similar to that observed in other causes of aplastic anemia, further supports the possibility that exposure to benzene increases the risk of developing acute leukemia (U.S. EPA, 1977).

Delore and Borgomano (1928) first described the association between benzene exposure and leukemia in a worker exposed to benzene for 5 years, who developed acute lymphoblastic leukemia. In 1939 two cases of leukemia among patients who had chronic benzene exposure in the industries around Boston were described (Bowditch and Elkins, 1939; Hunter, 1939; Mallory, et al. 1939). One patient had been exposed to benzene for 10 years, 4 years heavily (200 ppm) and the succeeding 6 years lightly, but had displayed hematologic evidence of benzene intoxication from the beginning of his employment. In the last 3 months of his life, the typical pattern of an acute myeloblastic leukemia developed. The characteristic findings of leukemia were found at autopsy which included diffused myeloid infiltration of the liver, spleen, and bone marrow. The other case was a 12-year-old boy, a painter's son, who used his father's paint shop to repaint toys, using a paint remover known to contain benzene. He developed aplastic anemia but sternal puncture and sternal biopsy revealed a typical leukemia replacement of the marrow with undifferentiated cells of the lymphoblastic series. DeGowin (1963) reported a case involving a painter who had been exposed to benzene for 13 years. He developed a hypocellular bone marrow and pancytopenia, followed by a relatively normal bone marrow with variable leukopenia, anemia, and thrombocytopenia. Then after 15 years a distinctly leukemic marrow and pancytopenia were found. Tareeff, et al. (1963) described six acute and 10 chronic leukemia cases in workers in the U.S.S.R. occupationally exposed to benzene for 4 to 27 years. In three of the acute cases a latent period of 2 to 5 years from cessation of exposure was noted.

Although case reports have suggested that benzene causes leukemia, convincing evidence has come from epidemiological studies. Many of these have come from Aksoy and his colleagues in Turkey (Aksoy, 1977; Aksoy, et al. 1972b, 1976a,b, 1977a,b). They described individual case reports of workers with aplastic anemia progressing through a preleukemia phase to acute myeloblastic leukemia or erythroleukemia; an accumulation of cases resulting in a statistically significant higher incidence of acute leukemia among shoe workers; and an outbreak of leukemia in this population that appears temporally related to the onset of benzene use and that has subsided following replacement of benzene as a solvent for adhesives.

Aksoy, et al. (1974b) observed 26 cases, during the period 1967-73, of acute leukemia among 18,500 shoe workers exposed to maximums of 210 to 650 ppm benzene for 1 to 15 (mean 9.7) years. Fourteen cases were acute myeloblastic leukemia, four preleukemia, three acute erythroleukemia, three acute lymphoblastic leukemia, one acute promyelocytic leukemia, and one acute monocytic leukemia. From these data they derived a leukemia incidence of 13 per 100,000, which is statistically significantly higher than the risk of 6 per 100,000 assumed for the general population. The latter figure is derived from leukemia incidence in more developed countries than Turkey and thus may be high. Aksoy (1977) recently estimated the incidence of leukemia in the general population of Turkey as 2.5 to 3.0 per 100,000. Moreover, if the relative incidence were computed solely for acute myeloblastic leukemia and its variants, a magnification of the risk in benzene-exposed shoe workers would be observed. Secondly, in their series the average age at the diagnosis was 34.2 years. This is a relatively low-risk age period for leukemia, with a reported death rate about half of the overall incidence (Cooke, 1954). Recalculation of their data with an age factor would presumably increase the statistical significance of the findings (U.S. EPA, 1977). Thirdly, Aksoy (1977) believes that shoe workers with acute leukemia were probably admitted to other Istanbul hospitals without his knowledge.

Aksoy, et al. (1976b) reported that, of 34 patients, six exposed to 150 to 210 ppm benzene vapor for up to 28 years (mean exposure: 11 years) were diagnosed as having Hodgkin's disease. Twenty other patients also exposed to benzene were later diagnosed as leukemics. Based on case studies, the authors stated that benzene, because of its toxicity to both the hematopoietic and reticuloendothelial system, is etiologically related to the onset of Hodgkin's disease. Alternatively, the authors proposed that benzene may act with other unknown factors contributing to the onset of this proliferative disorder.

Aksoy (1977) presented his observations of acute leukemia in shoe workers for the period 1967-76. These annual incidence data appear below:

1967	1	1972	5
1968	1	1973	7
1969	3	1974	4
1970	4	1975	3
1971	6	1976	0

The peak incidence (19.7 per 100,000) of leukemia in shoe workers occurred between 1971 and 1973. This follows by a few years the appearance of a notable incidence of aplastic anemia in this occupational group. The decline in cases since 1973 is temporally related to a decrease in use of benzene as an adhesive solvent, which began gradually in 1969. Aksoy also reports that pancytopenia was present in 27.5 percent of the cases before the onset of acute leukemia, which occurred 6 months to 6 years later. The hematological findings often indicated a period of recovery before the onset of leukemia, a phenomenon also noted by other investigators. Aksoy states that, during this period, over 100 cases of aplastic anemia were observed that were either idiopathic or associated with an agent other than benzene, and in none of these cases did acute leukemia develop. He also states the opinion that no blood dyscrasia is required before the other onset of leukemia and provides an example of a 23-year-old shoe worker who was hematologically normal when studied 4 years before the onset of acute erythroleukemia.

Vigliani and Saita (1964) estimated the number of workers exposed to benzene in northern Italy, and, based on the incidence of acute leukemia in the general population of Milan, calculated a 20-fold higher risk of acute leukemia in these workers. More recently Vigliani and Forni (1976) summarized their experience from 1942 to 1975. During this period they observed 66 cases of significant benzene hematotoxicity in Milan, mostly in shoe workers; 11 of these were acute myelogenous leukemia. In Pavia during the period 1959-74 they observed 135 shoe workers with benzene hematotoxicity, 13 with acute myelogenous leukemia. Benzene concentrations were usually 200 to 500 ppm. They also observed two cases of myelogenous leukemia in the rotogravure industry where ambient benzene exposures were calculated to be 200 to 400 ppm, with peaks up to 1,500 ppm.

Ishimaru, et al. (1971) performed a retrospective study of survivors of the two atomic bombings of Japan, evaluating the effects of occupation on the incidence of leukemia. Two occupations were considered to involve exposure to benzene, and these occupations taken together were associated with an increased risk for leukemia (30 cases, 14 controls, relative risk = 2.3 $p \leq 0.01$). Twenty-four leukemia cases were too far from the atomic bomb explosion for radiation to have influenced the increased risk. The increased risk, however, could be associated with exposures other than to benzene, as in none of the 10 occupations considered would benzene be the only chemical encountered. The risk was significantly higher in those with 5 or more years of potential exposure but not in those who had been employed in such occupations for less than 5 years. The relative risks were similar in Hiroshima and Nagasaki and were higher for acute leukemia (2.9) than for chronic leukemia (1.8).

Girard and Revol (1970) evaluated the frequency of a positive history of benzene exposure in 401 patients hospitalized with serious hematological disorders, compared with 124 patients hospitalized for other reasons. A statistically significant increase in history of benzene exposure was found in patients with aplastic anemia (10/48, 21 percent), acute leukemia (17/140, 12 percent), and chronic lymphocytic leukemia (9/51, 15 percent) compared with the control patients (5/124, 4 percent).

The Occupational Health Studies Group of the University of North Carolina has studied the health status of rubber-industry workers, a group exposed to various solvents including benzene (Andjelkovic, et al. 1976, 1977; McMichael, et al. 1974, 1975, 1976a,b; Tyroler, 1977). They have evaluated the 10-year mortality experience of a large cohort of male workers (5,106 deaths) at four tire manufacturing plants. The subjects were in the work force or were retired in 1964. The mortality due to all cancers (1,014) was normal or slightly elevated, depending on the data base used for comparison. Deaths due to cancer of the lymphatic and hematopoietic system (total of 109) were 31 percent higher than expected and were increased in cohorts of each of the four companies. In the category of lymphosarcoma and Hodgkin's disease, the standard mortality ratio (SMR) was 129 and an increase in the expected number of deaths was observed in two of the four company cohorts. Similarly, for deaths due to all forms of leukemia the SMR was 130 and the increase was observed in three of the four cohorts. When this latter category was further subdivided, the overall SMR for lymphatic leukemia was found to be 158 and the expected death rate was elevated in two of the four company cohorts. Of particular note is that the SMR for deaths attributed to lymphatic leukemia was 291 in the age group 40 to 64.

Several approaches were followed in further study of the increased incidence of lymphatic leukemia. Contrasting the work history of 17 patients with lymphatic leukemia with those of three matched controls for each case revealed that solvent exposure increased the overall risk by a factor of 3.25. Further classifying the groups according to high, low, and medium solvent exposure yielded a 5.5 factor for the high-exposure group. In those patients first subjected to high exposure between 1940 and 1960, the factor for the relative risk of lymphatic leukemia was 9.0. The relationship of solvent exposure to lymphatic leukemia was statistically significant at $p \leq 0.025$. The study also showed an increase in the mean difference in years of work history between lymphatic leukemia and the case controls. This was inversely proportional to the extent of solvent exposure. A limitation is the lack of historical data concerning the benzene exposure or the concentrations of other solvents, e.g., xylene, toluene, and trichloroethylene. These studies do, however, strongly support the possibility that long-term exposure to benzene in the U.S. rubber industry leads to an increased risk of lymphatic leukemia (U.S. EPA, 1977).

Monson and Nakano (1976a,b) evaluated a cohort of 13,571 white male rubber workers and found an SMR of 128 for leukemia.

Thorpe (1974) surveyed 38,000 workers from eight European affiliates of a major petroleum company to determine if there were differences in leukemia incidence rates between workers in occupations in which there was possible benzene exposure (such as refinery workers) and those in occupations where there was no exposures (office workers). Leukemia incidence rates were determined over a ten-year period from 1962 to 1971. As there were no benzene sampling data, actual exposures were unknown, and the workers were grouped on the basis of "potential exposure" to benzene, determined by the nature of their work. The data obtained revealed no statistically significant differences in leukemia incidence rates between exposed workers and standard age-adjusted populations. Thorpe (1974) reported increased, but not statistically significant, incidence rates in benzene-exposed workers when compared with the nonexposed workers in the study. The case-finding techniques used by Thorpe (1974) and the reliability of the number of reported leukemia cases were criticized by Brown (1975).

The most convincing epidemiological study implicating benzene as a leukemogen is the recent one by Infante, et al. (1977a) from the National Institute for Occupational Safety and Health. They followed for vital status, up to mid-1975, 748 white males exposed to benzene in the manufacture of a rubber product from 1940 through 1949. A statistically significant ($p \leq 0.002$) excess of leukemia was found in comparison with two control populations, the general American population, and another industry not using benzene. There was a fivefold excessive risk of all leukemias and a tenfold excessive risk of myelocytic and monocytic (probably myelomonocytic) leukemias combined. The single case of chronic myelocytic leukemia had a lag period of 2 years from initial benzene exposure, but the six cases of acute myelocytic and monocytic leukemia had lag periods of 10 to 21 years. The true leukemia risk to benzene-exposed workers was thought to be much higher because the follow-up of the study population was only 75 percent complete, and the remaining 25 percent were all regarded, in the calculations, as being alive at the end of the study period. The environment of the workers was not contaminated with any solvents other than benzene, and benzene concentrations in the air were generally below the recommended limit in effect during the period of the study, i.e., 100 ppm (1941), 50 ppm (1947), 35 ppm (1948), 25 ppm (1957), and 10 ppm (1969). In general, this epidemiological study provides excellent confirmatory evidence of the causal relationship of benzene exposure to acute myelocytic leukemia (U.S. EPA, 1977).

Ott, et al. (1978) recently studied the mortality experience of 594 workers exposed to benzene in the chemical industry. The workers were stratified by benzene exposure levels, and hematological findings were carefully examined. The cause-specific mortality rates for the 102 deceased individuals agreed well with those observed in a study of over 8,000 other employees in the same area. No association with benzene exposure was detected; however, two deaths due to acute myelogeneous leukemia, and one with myeloblastic leukemia listed as a significant associated condition, occurred. The time-weighted average benzene exposure of these three individuals was below 10 ppm. The expected number of myelogenous leukemia cases in this study population is 0.8, and the observed number of three is only of marginal statistical significance. These inconclusive findings may be due to the small number of deaths evaluated in the study (U.S. EPA, 1977).

With reference to the quantitative relationship of benzene exposure level to the development of acute leukemia, the available literature is inadequate for the generation of dose-response curves (U.S. EPA, 1977). In contrast with pancytopenia, where a large percentage of benzene-exposed individuals have developed benzene hematotoxicity and thus the available monitoring information might be used to estimate the average benzene exposure, leukemia occurs in a very small percentage of the benzene-exposed, and those developing leukemia may have been exposed to higher concentrations than indicated by area-wide monitoring systems. This exposure might occur because of the specific job involved or faulty work habits, e.g., failure to wear a respirator. In those studies of acute leukemia where benzene exposure levels have been reported, the concentrations have generally been above 100 ppm (Aksoy, et al. 1972, 1974a,b, 1976a,b; Vigliani and Forni, 1976; Vigliani and Saita, 1964; Kinoshita, et al. 1965; Sellyei and Kelemen, 1971). The often-reported longer period of benzene exposure required for the development of acute leukemia than for pancytopenia might well be a spurious consequence of the frequent lag period between the initiation of benzene exposure and the development of acute leukemia.

Also reported in association with benzene exposure have been lymphosarcoma (Bousser, et al. 1948; Caprotti, et al. 1962), Hodgkin's disease (Aksoy, et al. 1974c; Mallory, et al. 1939), reticulum cell sarcoma (Paterni and Sarnari, 1965), and multiple myeloma (Tareeff, et al. 1963; Torres, et al. 1970), but none of these case reports suggests other than a chance relation to benzene exposure (U.S. EPA, 1977).

CRITERION FORMULATION

Existing Guidelines and Standards

Existing air standards for occupational exposure to benzene include 10 ppm (32 mg/m^3) and an emergency temporary level of 1 ppm by the U.S. Occupational Safety and Health Administration (NIOSH, 1974, 1977), 25 ppm (80 mg/m^3) by the American Conference of Governmental Industrial Hygienists (ACGIH, 1979), 16 ppm promulgated by Czechoslovakia in 1969, and 6 ppm (20 mg/m^3) promulgated by the Soviet Union in 1967. OSHA also prohibits repeated or prolonged skin exposure to liquid benzene. No standard for benzene in water exists, but Cleland and Kingsburg (1977), using several assumptions and ACGIH air standards, have suggested values of 1,071 and 414 µg/l for ingested water, and 107 µg/l for ingested water based on the potential carcinogenicity of benzene.

Current Levels of Exposure

As discussed previously under "Exposure," the major source of human exposure to benzene is through the respiratory route. The annual average exposure of an individual to ambient benzene from all air sources is 1.03 ppb (Mara and Lee, 1977).

The U.S. EPA (Mitre Corp., 1978) has attempted to put into perspective the known and unknowns about total benzene exposure for its National Drinking Water Program. Based upon the assumptions utilized, air was the predominant source of benzene absorbed by the general population. This source contributed more than 80 percent of the total daily benzene uptake for an adult male living in an urban environment. Assumed benzene content in drinking water included levels of 0.1, 0.2, 1.0, and 10 µg/l, food was 250 µg/l, and ambient air was 50 µg/m^3. The total daily intake at the 10 µg/l benzene level for drinking water was 1.128 mg/day of which 1.4 percent came from the water, 17.7 percent came from food, and 80.9 percent came from ambient air exposure.

As shown by Mara and Lee (1977) certain occupational groups have potential exposure to benzene over and above the ambient levels. The representative industry activities include chemical manufacturing, coking operations, gasoline service stations, petroleum refineries, and solvent operations.

Special Groups at Risk

There is some suggestion that there may be genetic predisposition to benzene toxicity; this subject is reviewed by Goldstein (1977b). Although there are many more cases of benzene-induced hematotoxicity in males than in females because of occupational exposure, there is evidence to suggest that exposed females have a greater chance of developing severe disease (Mallory, et al. 1939; Ito, 1962). Age does not seem to affect hematotoxicity (Aksoy, et al. 1971).

Basis and Derivation of Criterion

The NAS (1977), in its review of drinking water and health, concluded that existing animal and human data did not allow the establishment of limits for benzene in drinking water. This was because the animal results were not statistically significant and were based on nonoral administration of benzene. In addition, the occupational studies on human exposure did not contain adequate information on degree of exposure or size of the population at risk, and did not rule out exposure to other chemicals besides benzene. However, most significant findings of Maltoni and Scarnato (1979) and those of Goldstein, et al. (1980) provided strong evidence for leukemogenic activities of benzene in Sprague-Dawley rats. Furthermore, prevalence of other types of tumors were observed (Tables 4 and 5).

Since the publication of the NAS report, the a[illegible]e-described epidemiological studies by Aksoy (1977), Infante, et al. (1977), and Ott, et al. (1978) have appeared. These studies include information on degree of benzene exposure and size of the population at risk, and rule out exposure to solvents other than benzene. The U.S. EPA Carcinogen Assessment Group (1978a) has made use of these three occupational studies to calculate a leukemia dose-response curve. The slope of this curve is 0.024074, in units of lifetime risk of leukemia per ppm exposure to benzene in air. Since 1 ppm is 3.25 mg/m^3, and assuming a respiratory rate of about 20 m^3/day and a respiratory absorption coefficient Of 0.50, the benzene intake per individual at 1 ppm is:

$$(3.25\ mg/m^3)\ (20m^3/day)\ (.5) = 32.5\ mg/day$$

To calculate the benzene intake resulting in a lifetime risk of leukemia of 10^{-5}, one solves the following equation for x,

$$\frac{x}{10^{-5}} = \frac{32.5\ mg/day}{0.024074}$$

resulting in 0.0135 mg/day.

The U.S. EPA (Mitre Corp., 1978) total exposure analysis indicates that the total body exposure may be as high as 1.1 mg/day of benzene. This was derived using estimates which have varying degrees of support in terms of hard data. The specific use of the total exposure estimates for calculation of water criterion does not seem warranted at this particular time because of a general lack of knowledge about the accuracy of the estimates. It can be said, however, that from a general weight of evidence perspective, it appears that air exposure may contribute the majority of total exposure. The total exposure consideration should be factored into the criterion development at a later date when additional data is available.

Under the Consent Decree in NRDC v. Train, criteria are to state "recommended maximum permissible concentrations (including where appropriate, zero) consistent with the protection of aquatic organisms, human health, and recreational activities." Benzene is suspected of being a human carcinogen. Because there is no recognized safe concentration for a human carcinogen, the recommended concentration of benzene in water for maximum protection of human health is zero.

Because attaining a zero concentration level may be infeasible in some cases and in order to assist the Agency and states in the possible future development of water quality regulations, the concentrations of benzene corresponding to several incremental lifetime cancer risk levels have been estimated. A cancer risk level provides an estimate of the additional incidence of cancer that may be expected in an exposed population. A risk of 10^{-5} for example, indicates a probability of one additional case of cancer for every 100,000 people exposed, a risk of 10^{-6} indicates one additional case of cancer for every million people exposed, and so forth.

In the Federal Register notice of availability of draft ambient water quality criteria, EPA stated that it is considering setting criteria at an interim target risk level of 10^{-5}, 10^{-6}, and 10^{-7} as shown in the table below.

Exposure Assumptions (per day)	Risk Levels and Corresponding Criteria (1) µg/l			
	0	10^{-7}	10^{-6}	10^{-5}
2 liters of drinking water and consumption of 6.5 grams fish and shellfish. (2)	0	0.066	0.66	6.6
Consumption of fish and shellfish only.	0	4.0	40.0	400

(1) Calculated by applying a relative risk model for epidemiologic studies, as described in the Human Health Methodology Appendices to the October 1980 Federal Register notice which announced the availability of this document, to the human epidemiology data presented in Appendix I. Since the extrapolation model is linear at low doses, the additional lifetime risk is directly proportional to the water concentration. Therefore, water concentrations corresponding to other risk levels can be derived by multiplying or dividing one of the risk levels and corresponding water concentrations shown in the table by factors such as 10, 100, 1,000 and so forth.

(2) Two percent of the benzene exposure results from the consumption of aquatic organisms which exhibit an average bioconcentration potential of 5.21-fold. The remaining 98 percent of benzene exposure results from drinking water.

Concentration levels were derived assuming a lifetime exposure to various amounts of benzene, (1) occurring from the consumption of both drinking water and aquatic life grown in waters containing the corresponding benzene concentrations, and (2) occurring solely from consumption of aquatic life grown in the waters containing the corresponding benzene concentrations.

For comparison purposes the following risk estimate levels, as derived from experimental data in Sprague-Dawley rats (Zymbal gland carcinomas in females at the high dose) (Maltoni and Scarnato, 1979) and based on a modified "one-hit" extrapolation model as described in Federal Register (44 FR 15926), show remarkable similarities with the risk levels estimated from human epidemiological data as shown above (Aksoy, 1977; Infante, et al. 1977; Ott, et al. 1978).

Exposure Assumptions (per day)	Risk Levels and Corresponding Criterion µg/l			
	0	10^{-7}	10^{-6}	10^{-5}
2 liters of drinking water and consumption of 6.5 g fish and shellfish.	0	0.12	1.2	12

Although total exposure information for benzene is discussed and an estimate of the contributions from other sources of exposure can be made, this data will not be factored into ambient water quality criteria formulation until additional analysis can be made. The criteria presented, therefore, assume an incremental risk from ambient water exposure only.

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Appendix

Derivation of Criterion for Benzene

Three epidemiology studies of workers exposed to benzene vapors on their jobs, performed by Infante, Ott, and Aksoy, were reviewed by the CAG for the Office of Air Quality Planning and Standards (Albert, 1978). Their result was that the potency for humans breathing benzene continuously is $B = 0.02407$. This means that the lifetime risk of getting leukemia, R, equals 0.024074 times the lifetime average continuous exposure, X, measured as ppm of benzene by volume in air, or $R = B\ X$. Therefore the air concentration, X, resulting in a risk of 10^{-5} is $X = R/B = 10^{-5}/.024074 = 4.1539 \times 10^{-4}$ ppm.

Since the air concentration corresponding to 1 ppm of benzene is 3.25 mg/m^3 and assuming a respiratory rate of 20 m^3/day and a respiratory absorption coefficient of 0.50, the daily intake that would result in a risk of 10^{-5} is:

$$4.154 \times 10^{-4}\ \text{ppm} \times 3.25 \times 10^{3}\ \mu g/m^3\ \text{per ppm} \times 20\ m^3/\text{day} \times 0.5 = 13.5\ \mu g/\text{day}$$

If it is assumed that the fraction of benzene absorbed is the same between inhalation and ingestion of water and fish, a daily benzene intake of 13.5 ug through drinking water and fish alone would also cause a leukemia risk of 10^{-5}. The water concentration given this intake is:

$$C = (13.5\ \mu g/\text{day})/(2 + 5.21 \times 0.0065)$$
$$= 6.64\ \mu g/l$$
$$= 6.6\ \mu g/l$$